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FOCUS

Plant growth regulators in water stress tolerance

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ABSTRACT

The present review provides an insight into the relationship between plant growth regulators and water stress with emphasis on metabolic events that regulate growth regulator balance and physiological responses. Possible mechanisms by which ABA controls stomatal function and growth under stress, and interacts with proteins and important osmo-protectants, have been discussed. ABA involvement in signal transduction and root-shoot communication through its effects on gene and gene products is also included. A brief description of involvement of other growth regulators such as cytokinins, ethylene, polyamines and brasssinosteroids in water stress tolerance is also provided. Salient achievements in exploiting the potential of growth regulators in the resistance to water stress in some horticultural crops are also given. Gaps in existing information on plant growth regulator research in water stress tolerance have been summarized.

Key words: Abscisic acid, brassinosteroids, cytokinins, ethylene, polyamines, water stress

INTRODUCTION

Water deficit stress is a serious and frequently encountered abiotic stress in the terrestrial surface. Its deleterious effects on plant growth and productivity are well documented. Plant responses to water stress are believed to be complex as these operate at various levels of plant organization. Several in-built physiological and biochemical mechanisms provide resistance to plants against stress. An understanding of the processes linked to these mechanisms is vital for optimizing crop growth and productivity under stress.

Plants respond and adapt to water stress by altering cellular metabolism, thus invoking stress tolerance. Alteration in endogenous concentrations of growth regulators along with accumulation of osmolytes, modifications in antioxidant cascade, changes in protein profiles and induction of gene expression in plants under stress are important characteristic metabolic changes that invoke stress tolerance at the cellular level. Alteration in endogenous concentrations of growth regulators under stress helps plants through better turgor maintenance and efficient water usage by influencing stomatal functioning, hydraulic conductivity and morphological adaptation (Fig 1). Progress made in plant adaptation to water stress is an outcome of advances made in analytical techniques on endogenous growth regulator analysis, and, powerful and reliable molecular and genetic techniques.

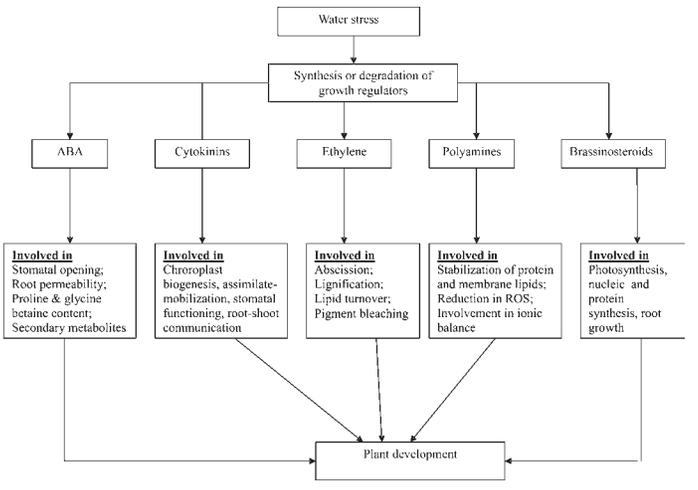

Fig 1. Water stress induced response of growth regulators in plants

The aim of this review is to provide comprehensive information on physiological, biochemical and molecular aspects of growth regulators in stomatal control, signal transduction, induction of proteins and gene expression under water stress. Because of the vast pool of information, emphasis is laid on abscisic acid (ABA). A brief account of other growth regulators such as cytokinins, ethylene, polyamines and brassinosteroids involved in stress tolerance

is also provided. Studies on the use of growth regulators for amelioration of water stress in some horticultural crops are also included.

a) Abscisic acid

i) ABA biosynthesis and accumulation

Water stress affects ABA biosynthesis, leading to its accumulation. Evidence for ABA biosynthesis has been obtained by radio label ^{18}O experiments, molecular genetic analysis of auxotrophs and biochemical studies. ABA biosynthesis takes place in the cytosol through the carotenoid biosynthetic pathway (Milborrow, 2001) (Fig 2). Zeaxanthine, produced after cyclization and hydroxylation of *trans*-lycopene via β-carotene, is converted into violaxanthine (Nambara and Marion-Poll, 2005). 9-*Cis*-epoxy carotenoid dioxygenase (NCED) enzyme cleaves violaxanthine to a C_{15} product, *cis*-xanthoxine, and a C_{25} metabolite (Schwartz *et al*, 2003). The ABA is produced from *cis*-xanthoxine via the intermediate abscisic aldehyde through involvement of the enzyme abscisic aldehyde oxidase. During water stress, activities of the enzymes associated with of biosynthesis ABA and relative mRNA are induced in abundance in leaves/roots. Inhibition of catabolism of ABA is also important in stress-induced ABA accumulation. ABA is catabolised in plants into its hydroxylated products, phaseic acid (PA) and dihydrophaseic acid (DPA) (Zhou *et al*, 2004) or converted into the physiologically inactive glucose ester (Boyer and Zeevaart, 1982). Studies have revealed that PA and DPA levels increase in parallel to ABA. However, their levels under stress increase even after the ABA content has reached a plateau. In contrast, upon rehydration of plants, ABA level shows a decrease but PA or DPA levels either increase or remain unaltered. Jia and Zhang (1997) stated that inhibition of ABA catabolism also contributed to ABA accumulation in plants under stress. However, there is no evidence for ABA release from esters under stress.

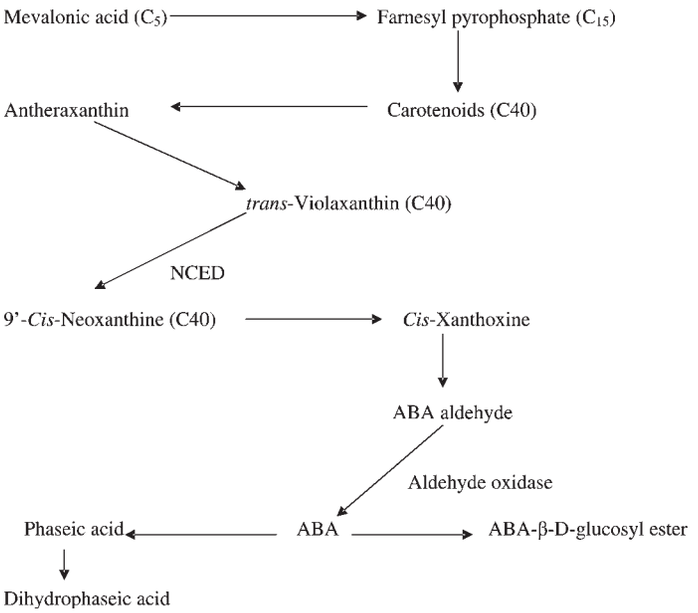

Fig 2. Biosynthetic pathway of ABA (Milborrow, 2001)

Water stress substantially accumulates ABA in a number of plant species including horticultural crops such as tomato, French bean, onion, etc. The enzyme NCED is proposed to be a key enzyme in ABA accumulation (Qin and Zeevaart, 2002). The amount accumulated depends upon factors such as severity of stress, cultivar, species, tissue and the developmental stage. The increased ABA content plays an important role in stress tolerance following its action on stomatal regulation, root-shoot communication, induction in stress proteins and associated genes, osmolyte synthesis, senescence-promotion thereby reducing plant water use, and on maintenance of the antioxidant pool. ABA concentrations are considered a vital tool in selection and breeding of varieties for drought tolerance.

ii) ABA-induced stomatal regulation under water stress

Drought stress induces stomatal closure in the leaves of many plant species. Using this mechanism, plants are able to restrict water loss through transpiration. This response is associated with decline in leaf turgor and/or water potential (Maroco *et al*, 2002). Further, this regulatory mechanism is found to be linked more to the soil moisture content than to leaf water status, thereby suggesting that stomata are responsive to chemical signals produced by dehydrating roots (Davies and Zhang, 1991). Sensitivity of the stomata to ABA varies widely in different species and cultivars, and, is dependent upon leaf-age, temperature, ambient CO_2 concentration, plant nutritional status, ionic status of xylem sap and leaf-water status (Dodd *et al*, 1996). Differences in stomatal response to ABA may be a consequence of differences in the quantity of ABA reaching the active site in the guard cell. Xylem ABA concentration and stomatal conductance showed linear inverse relationship, and the scope of relationship varied diurnally with the most sensitive stomatal closure recurring at lower water potential (Tardieu and Simmoneau, 1998).

The stomatal aperture is regulated by turgor potential of surrounding cells. The guard cell volume is actively responsive to signals produced under stress in order to regulate CO_2 efflux for photosynthesis and transpirational

water loss. The ABA increase in guard cells reduces plant water loss through transpiration by promoting stomatal closure (Harris and Outlaw, 1991). The influx or efflux of K^+, balanced by flux of anions in the guard cell, regulates guard cell volume (Hetherington and Quatrano, 1991). MacRobbie (1991) showed that externally applied ABA evoked efflux of K^+ and anions from the guard cells. Blatt (1990) found very rapid activation of K^+ channel by ABA. Rapidity of this response and lack of modulation by other cytoplasmic factors suggest that ABA is activating this channel directly. Progress is also made in deciphering electrical responses triggered by ABA in the plasmalemna of guard cells (Blatt and Theil, 1993, MacRobbie, 1997, Schoeder, 1992). The cellular electrical changes induced by ABA are an outcome of the depolarization effect which reflects a net influx of cations (Thiel *et al*, 1992). Depolarization is the driving force for K^+ efflux through outward K^+ channel.

Ca^{+2} play an important role in ABA-mediated stomatal closure. Ca^{+2} participate as an intracellular secondary messenger in mediating ABA effects on stomatal aperture and/or plasma membrane channel. ABA is shown to induce an increase in guard cell Ca^{+2} concentrations, which precedes stomatal closure (Irving *et al*, 1992). ABA is also shown to evoke alkalization of the cytoplasm of guard cells (Irving *et al*, 1992), which is necessary in ABA activation of the K^+ channel (Blatt and Armstrong, 1993). The ABA–induced rise in internal Ca^{+2} concentration is contributed by an influx of external Ca^{+2} as well as Ca^{+2} released from intracellular stress (Gilroy *et al*, 1991, McAinsh *et al*, 1991). Inositol 1, 4, 5 – triphosphate is an essential intermediate for triggering cellular Ca^{+2} mobilization.

Protons can directly affect stomatal aperture and/or its sensitivity to ABA. Maintenance of optimum apoplastic pH for stomatal opening is vital for stomatal activity (Wilkinson and Davies, 1997). Feeding artificial sap of pH 7.0 to intact leaves of ABA deficient tomato mutant *Flacca* increased stomatal aperture and transpirational water loss compared to feeding sap buffered to pH 6.0 (Schwartz *et al*, 1994). Other studies also shown revealed that reduced pH sensitizes stomata to ABA (Anderson *et al*, 1994) as guard cells take up ABA more efficiently at more acidic pH and its receptivity to internally located molecular receptors is enhanced.

Patonnier *et al* (1999) gave evidence for involvement of apoplastic sugars in deciding guard cell sensitivity to ABA. There is an increase in the concentration of apoplastic sugars with reduction in soil water potential, concomitant with a decrease in stomatal conductance. Effects of sugars on stomata are specifically on an increase in the anion efflux channel activity of the guard cell. As ABA also induces anion loss and reduces turgor in guard cell, it is imperative that sugars and ABA act synergistically in closure of stomata (Hedrich and Morten, 1993).

Recent studies have depicted H_2O_2 as an important stress signal transduction molecule promotory to stomatal closure (Luan, 2002). Zhang *et al* (2001) showed that ABA increases H_2O_2 production.

iii) Root to shoot communication and involvement of ABA

Several investigators have reported that shoot growth is more inhibited in plants experiencing water stress than is root growth (Munns and Sharp, 1993, Passioura and Gardner, 1990, Sauter *et al*, 2001). Some studies have also found faster root growth in limited soil water environment (Munns and Sharp, 1993). Inhibition of shoot growth and increase in root weight under stress cannot be explained in terms of reduction in photosynthesis, water or nutrient supply.

Investigations have revealed the association of ABA in the process by which root weight increases in response to water stress (Blackman and Davies, 1985, Carmi and Heuer, 1981, Zeevaart *et al*, 1991). Creelman *et al* (1990) and Robertson *et al* (1990) showed that exogenous ABA application caused greater reduction in shoot growth than in root growth. Evidences of sustained increase in root growth have also been found (Biddington and Dearman, 1982; Watts *et al*, 1981). Mutant research also depicted a role for ABA in differential regulation of shoot and root growth. Saab *et al* (1990) reported that ABA-deficient roots grew more slowly at low water potential than the normal ones, while, shoots grew faster. Sharp *et al* (1994) reiterated that exogenous ABA application to ABA deficient plants led to increase in root growth.

The ratio between root and shoot is sensitive to environment and there is coordination among the two via long-distance transport of substrates or through a signal (Munns and Crammer, 1996). Passioura and Stirzaker (1993) opined function of feed-forward signals under adverse soil conditions. In feed-forward controls, plants sense the environment and communicate the status to other plant parts by a signal, and can also provide advance warning of a changing environment. Roots sense soil conditions and send

signals to leaves that slow down growth before supply of water/nutrients becomes limiting. The feed-forward signal from roots to the aerial plant parts under water stress is demonstrated to be operating through ABA. Jackson (1993) provided evidence for influence of roots on shoot development via transport of hormones in the xylem.

ABA moves readily in the phloem (Hoad, 1995). It is found in substantial quantities in the phloem exudate, and increases rapidly in plants exposed to soil water deficit (Hoad, 1995). The function of phloem ABA is unclear. Hoad (1975) and Lovey (1984a), employing radio tracer techniques, observed that the ABA synthesized in leaves appeared later in roots and xylem sap. This suggested the possibility of ABA translocation from leaves. Munns and Crammer (1996) suggested that turgor reduction in leaves, under the influence of water stress and thus induced ABA levels, would cause recirculation of ABA in phloem and xylem sap resulting in promotion of early stomatal closure to prevent turgor loss.

Under stress, there is increase in xylem ABA concentration concomitant with reduction in leaf growth (Zhang and Davies, 1990, Hartung *et al*, 1994). Munns (1990) observed a direct relationship between xylem ABA increase and decline in leaf growth. However, Jackson (1993) concluded that ABA in xylem sap was not associated with leaf growth reduction. Munns (1992), using exogenous feeding of ABA to detached shoots at concentrations equivalent or greater to that found in sap of intact plants, observed significant reduction in leaf area at a concentration not found in the intact plant in drying soil. However, this response is species-dependent (Dodd and Davies, 1996, Munns and King 1988, Hartung *et al*, 1994, Bano *et al*, 1993).

ABA action on root are different from that in the shoot. Root expansion is often inhibited by exogenous application of ABA (Barlow and Pilet, 1984, Crammer and Jones, 1996). However, there are contrasting reports of ABA stimulating root growth (Biddington and Dearman, 1982; Watts *et al*, 1981). Saab *et al* (1990) observed that the relationship between ABA and root growth is completely different from that in shoot, in that, higher ABA levels improve rather than reduce root growth at low water potential. Glinka and Reinhold (1971) reported that ABA increased the flow of water by increasing the hydraulic conductivity of roots and enhancing ion uptake, which caused an increase in the water potential gradient between soil and root. External application of ABA increased water-absorbing area of the root which helped the plant to cope with drought conditions. Gaither *et al* (1975) revealed that ABA stimulated growth of excised root tips. Contrasting effects of ABA seen on roots and shoots may be due to differences in receptors, compartmentation control or interaction effects with other molecules. However, this aspect needs further investigation.

ABA involvement in the inhibition of cell expansion in leaves of water stressed plants indicates that ABA is an essential component of long-distance signaling pathway from root to shoot. The pathway may or may not involve interaction with other growth regulators. This suggests the possibility of xylem sap containing compound(s), preferably precursors of ABA or ABA-unrelated compounds, that stimulate the rate of ABA synthesis in growing cells (Dodd and Davies, 1996, Munns, 1992). Other growth regulators, such as cytokinins (Stoll *et al*, 2000) and ethylene (Hussain *et al*, 2000), may act in concert with ABA, either in the xylem sap or in growing cells.

iv) Signal transduction

Signal transduction is molecular description of the regulatory network that relates perception of signal to cellular response (Fig 3.). Interesting researches have been made in signal transduction processes from sensing drought stress signal to the expression of various genes. Regulation of stomata is a well-described response of plants to water stress (Harris and Outlaw, 1991, Kearns and Assmann, 1993). ABA regulates stomatal aperture by promoting stomatal closure or by inhibiting stomatal opening through induction of changes in osmotic potential, mechanical properties of guard cells, or gene expression (Hetherington, 2001). Phosphorylation processes and protein kinases are thought to have an important role in signal transduction cascades in plants (Redhead and Palme, 1996).

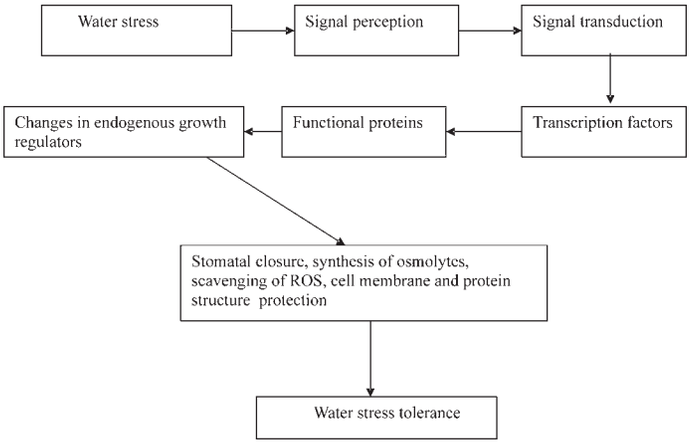

Fig 3. Sequence of events associated with water stress tolerance

ABA-dependent stomatal regulation under stress has also been shown to operate through involvement of cytoskeleton reorganization (Luan, 2002) by virtue of changes in elastic properties of guard cells. Eun and Lee (1997) reported changes in reorganization of the actin structure of guard cell. Guanosine triphosphate protein *AtRac1* was identified in *Arabidopsis* as central component in ABA-mediated disruption of the guard cell actin cytoskeleton (Lemichez *et al* 2001). ABA signal can also be transmitted to the guard cell nucleus to alter the pattern of gene expression, leading to alteration in profiles of protein involved in water transport, ion transport, or carbon metabolism (Dodd *et al*, 1996, Pospisilova and Dodd, 2005). Besides, several genes encoding G-protein, protein kinase and the transcription factor involved in signal transduction pathways are induced by ABA as also by water stress (Palme, 1993).

Mutant research has given important information on ABA involvement in water stress signal transduction pathways (Giraudat *et al*, 1994). *VP1* and *abi1*, *abi2* and *abi3* mutants have been extensively characterized and genes cloned. Among them, the ABI 1 gene product functions in stomatal closure and acts as a negative regulator of ABA–dependent gene expression. The dehydration-inducible ATCDPK1 encoding CDPK, by contrast, functions as a positive regulator. Thus, protein phosphorylation and dephosphorylative processes might be involved in ABA-responsive signaling during stress. In aleurone protoplast, MAPK is induced actively by ABA. A relationship between ABA-induced MAPK activation and ABA-induced gene expression showed involvement of MAPK in signal transduction.

v) Induction of osmo-protectants by abscisic acid

Water stress is found to increase the concentration of compounds (such as proline and glycine betaine in plants) that help in reducing cellular injuries via modifications in the osmo-regulation process. Free proline acts as an important osmo-protectant (Handa *et al*, 1983, Yoshiba *et al*, 1997, Heuer and Nadler, 1998) and as a storage compound for reduced carbon and nitrogen during water stress (Hare *et al*, 1998). Accumulation of proline in the leaves under stress is an important plant adaptation process. Exogenous ABA is shown to up-regulate proline biosynthesis in plants experiencing water stress (Stewart 1980, Ober and Sharp, 1994). Stewart (1980) showed that the metabolic cause of ABA-induced proline is a consequence of stimulated proline biosynthesis from glutamic acid. Pesci (1987) stated that the inhibition of utilization of precursor(s) of proline for protein synthesis does not contribute to proline accumulation by ABA. Verslues and Bray (2005) reported that ABA deficient mutants had less ability to accumulate proline. Applied ABA can also induce proline accumulation in turgid leaves and ABA accumulation precedes that of proline in wilting leaves. Proline is synthesized from glycine via the involvement of an enzyme pyrroline-5-carboxylate synthetase (P5CS) (Yoshiba *et al*, 1997). Savoure *et al* (1997) and Yoshiba *et al* (1997) reported induction of expression of P5CS gene by stress and exogenous ABA both in wild-type and in ABA-deficient (abaI) and ABA insensitive (aba1 and aba2) mutants.

The other osmoprotectant which has gained prominence in ascribing plant tolerance to stress is glycine betaine. ABA is shown to increase its synthesis under water stress conditions (Unayayar *et al*, 2004, Gao *et al*, 2004). Increase in glycine betaine by ABA is found to be the result of induction of betaine aldehyde dehydrogenase enzyme (Gao *et al*, 2004). These observations reveal the enhancement of osmotic protectant pool in stressed plants as an alternate mechanism by which ABA copes with stress responses.

vi) ABA and stimulation of protein expression under water stress

Water stress induces metabolic alteration resulting in synthesis and/or accumulation of a wide range of proteins (Pareek *et al*, 1998, Bray, 1988, 1991, Bartels *et al*, 1996; Cohen and Bray 1990, Piatkowski *et al*, 1990, Plant *et al*, 1991, Yokota *et al*, 2002). An analysis of proteins provides insight into the complexity of stress-response and in stress tolerance mechanism (Ramgopal, 1987, Borkird *et al*, 1991). Studies have shown activation of some proteins by water stress as well as ABA and the information achieved has been useful in describing ABA involvement in cellular signaling processes in plant-stress interactions (Chandler and Robertson, 1994). Water stress alters translatable mRNA and protein species in many plant species. These include a group of small molecular weight proteins such as LEA (Late Embryogenesis Abundant), RAB (Responsive to ABA) and dehydrins (dehydration-induced proteins). Synthesis of ABA is the common dominant factor in induction of all these proteins.

LEA protein accumulates during the development of seed, with a correlative increase in ABA level (Skriver and Mundy, 1990). These proteins are present in the embryo until the seed starts germinating. Bartels *et al* (1996) showed

that LEA proteins can be induced in plants by desiccation stress or by treatment with ABA. One of the LEA proteins, α-amylase inhibitor, is induced by drought stress in embryos, concomitant with accumulation of ABA (Nedeva and Nikolova, 1997). Similarly, ABA-induced proteins were seen in aleuronic layers (Hong *et al*, 1992) and leaves and roots (Mundy and Chua, 1988) due to water stress and ABA. Close *et al* (1993) reported that D-11 family of LEA proteins is related to dehydration-tolerance and expression of most of these is found to be regulated by ABA (Hong *et al*, 1992). The ABA-deficient mutant of tomato showed no distinct ABA-responsive proteins when subjected to water stress, compared to the wild type (Bray, 1988). ABA treatment to *flacca* resulted in the synthesis of polypeptides similar to wild type. Studies of Cohen and Bray (1990) employing cDNA probes developed against three of the ABA responsive proteins confirmed the above findings. Singh *et al* (1989) showed that a low water potential environment is required for protein accumulation in response to ABA application. The stress responsive proteins have been thought to function in detoxification of cells during dehydration (Bartels and Sankar, 2005).

vii) ABA - regulated gene expression during water stress

A number of genes that respond to water stress at the transcriptional level have been found to be induced by ABA (Skriver and Mundy, 1990, Delasny *et al*, 1994). It appears that cellular dehydration induced by water stress triggers production of ABA which, in turn, induces expression of various genes. However, not all the genes induced by water stress are responsive to ABA. Thus, there is existence of ABA-dependent and ABA-independent signal transduction cascade between initial signal of stress and expression for specific gene. Genes expressed during stress help in protecting cells from stress injury by producing proteins involved in the signal transduction mechanism (Shinozaki and Yamaguchi-Shinozaki, 1997).

Genes under ABA control have been isolated from different plant species (Skriver and Mundy, 1990). Depending upon the way these have been isolated, the genes have been named either RAB or LEA genes (Galau *et al*, 1986, Mundy and Chua, 1988). These genes have been effectively used as a tool to develop molecular models of ABA action. ABA and water stress regulatory LEA genes have been cloned (Skriver and Mundy, 1990, Ingram and Bartels, 1996). These genes have been found to be transcriptionally regulated (Galau *et al*, 1986). The functions of gene products have been predicted from sequence homology with known proteins and are thought to play a role in protecting cells from water stress. *Cis* and *Trans* factors involved in ABA-induced gene expression have been analyzed extensively (Ingram and Bartels, 1996; Giraudat *et al*, 1994, Chandler and Robertson, 1996; Shinozaki and Yamaguchi-Shinozaki, 1997).

Several genes are induced by water stress in ABA-deficient (aba) and ABA-insensitive (abi) mutants. This revealed that these plants do not require ABA but do respond to it under conditions of stress (Chen and Gusta, 1983, Jayaprakash *et al*, 1998). Analysis of ABA inducible genes revealed that several genes require protein biosynthesis for their induction by ABA, suggesting that two independent pathways exist between ABA production and gene expression during stress. These pathways involve either ABA responsive gene expression or ABA dependent / independent gene expression. Some such genes are *rd29Alilti*, *kin1*, *Cor6.6lkin* and *Cor47lrd17* (Yamaguchi-Shinozaki and Shinozaki, 1993, Izawa *et al*, 1993). The promoter region of the *rd29A* gene was analyzed and a novel *Cis*-acting element responsible for dehydration was identified. A 9-bp conserved sequence, TACGACAT, termed as dehydration responsive element (DRE) is essential for regulation of dehydration-responsive gene expression. The DRE has been demonstrated to function as a *Cis*-acting element involved in induction of *rd29A* expression. DRE-related motifs have been reported in promoter regions of water stress inducible genes such as *Kin 1, Cor 6.6 and rab 17* (Nelson *et al*, 1996; Wang *et al*, 1995). This suggested that DRE related motifs are involved in drought-responsive but ABA-independent gene expression. Two independent families of DREB proteins, DREB1 and DREB2 have been reported to function as trans-acting factors in signal transduction pathways under water stress (Jin *et al*, 1998).

Many changes in mRNA levels observed during stress reflect transcriptional inactivation. Exogenous ABA can also induce these changes. Successes have been made in understanding of transcriptional control mechanisms of ABA and stress induction by identification of *Cis*-acting regulatory sequences and isolating the corresponding nucleotide sequences (Yamaguchi-Shinozaki *et al*, 1989, Iturriaga *et al*, 1996).

ABRE motifs are not involved in ABA regulated stress-inducible genes (Iwasaki *et al*, 1995). The distinct-sequence motif is essential for ABA response. Genes that are induced by ABA and encode other potential transcriptional factors include the box gene, ATHB-07, and

several myb homologous genes from *A. thaliana* and *C. plantagineum* (Nelson *et al*, 1996). Comparison of available promoter sequences of ABA and stress-inducible genes revealed that ACGT cores were conserved in many promoter elements of these inducible genes (Shen and Ho, 1996, Iturriaga *et al*, 1996). Existence of ACGT core sequence in the promoter region of these genes suggests that these genes may be mediated by ABA (Izawa *et al*, 1993, Busk *et al*, 1997). A 50-bp ABA responsive element (ABRE) is capable of conferring ABA inducibility. Many ABA-responsive genes contain more than one sequence element with an ACGT core. Involvement of these in ABA or stress-response needs to be investigated. The most efficient, characterized *Cis*-element is the one that contains CACGTC with the G-box ACGT core element (Shen and Ho, 1996). G-box related ABREs have been observed in ABA-responsive genes, though their function needs to be identified.

Several *bZIP* transcription proteins that respond to water stress and ABA treatment have also been identified and these are found to be involved in ABA-dependent pathway (Nakagawa *et al***,** 1996). Em gene is another ABA-responsive gene that has been found to accumulate in response to both ABA and water stress (Morris *et al*, 1990).

b) Cytokinins

Cytokinins are involved in many aspects of plant growth and development such as seed germination, apical dominance, photo-morphogenesis, chloroplast biogenesis, maintenance of assimilate mobilization, translocation and senescence, and in the regulation of stomatal functioning and root to shoot communication under stress. These are synthesized primarily in the roots (Chen *et al*, 1985, Binns, 1994), although some amounts can be synthesized by shoot apex and other plant tissues. Most of the naturally-occurring cytokinins are N^6- substituted adenosine molecules with branched five-carbon side-chain [Zeatin (Z) and isopentenyladenine]. The riboside derivatives and N- and O-linked glycosides of the free bases have also been identified and their biological activity established (Brzobohaty *et al*, 1994, Murti and Upreti, 2000, Binns, 1994). The two pathways for biosynthesis of cytokinins include *de novo* biosynthetic pathway (Chen and Melitz, 1979, Taya *et al*, 1978) and *t*RNA pathway (Skoog and Armstrong, 1970, Hall, 1970). The *de novo* biosynthetic pathway has been found associated for majority of the biologically active cytokinins. The key step in cytokinin biosynthesis is the formation of N^6- (Δ^2 – isopentenyl) adenosine -5'- monophosphate from Δ^2– isopentenyl pyrophosphate and adenosine-5'-monophosphate catalyzed by isopentenyl transferase (IPT) (Renske *et al*, 1992, Mok and Mok, 2001). In the other pathway, *t*RNA is degraded and isomerized to *cis*–zeatin by cis-trans isomerase (Mok and Mok, 2001). Cytokinins are irreversibly degraded by cytokinin oxidases to inactive products that lack the N^6-side chain (Brzobohaty *et al*, 1994, Galuszka *et al*, 2001). Cytokinin levels are also regulated in tissues via their O- and N- glucosylation conjugation reactions (Brzobohaty *et al*, 1994). Conn (1993) reported release of cytokinins from their O-glucosides by the action of specific β-glucosidase present in plants. Cytokinins have also been found to be regulated by other hormones, particularly, auxins (Dunleavy and Ladley, 1995).

Water stress leads to a decline in leaf cytokinin concentration (Naqvi, 1999, Pospisilova *et al*, 2000), although it is difficult to predict the actual changes in any specific cytokinin. Plants under water stress are known to exhibit reduced cytokinin concentration in the xylem sap and this response is usually rapid. Cytokinin activity returns to normal levels upon release of stress. The reduction is presumed to be a consequence of either reduced cytokinin biosynthesis, or enhanced degradation, or both.

Zhu *et al* (2004) reported that changes in the levels of Z and ZR (zeatin riboside) in the xylem sap of apple trunk depended upon drought cycles. During the first cycle of drought and rewatering, levels of Z and ZR in the sap of drought treated-trees decreased significantly, while, in the second, Z continued to decline but ZR did not change significantly. In the third cycle, there was no difference in Z concentration between drought treatments. Masia *et al* (1994) suggested that a decrease in cytokinins transport from root to shoot occurs during the onset of water stress. Pillay and Beyl (1990) reported reduction in cytokinin concentration in a drought-susceptible cultivar of tomato. Upreti *et al* (1998) and Upreti and Murti (2004a) reported

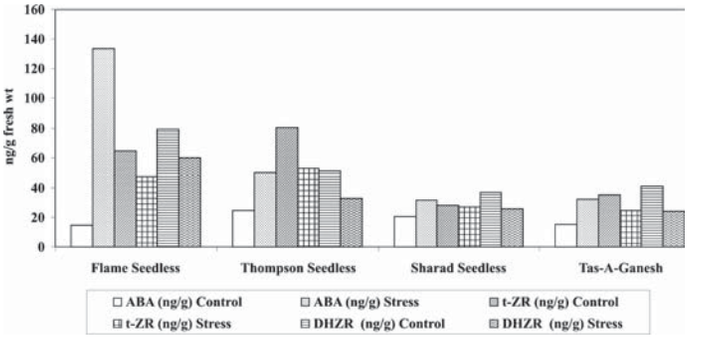

Fig 4. Influence of soil moisture stress on endogenous hormones in grape genotypes

a decline in levels of ZR and DHZR (dihydrozeatin riboside) in stressed leaves of French bean and onion. Satisha *et al* (2005) witnessed a decline in cytokinins in grape genotypes under soil moisture deficit conditions (Fig 4.). Upreti and Murti (2004b) observed that the decline in cytokinin under stress depended upon leaf-age with young leaves showing greater reduction. Water stress led to a decline in root nodulation in bean plants, which is linked to a decline in cytokinins in roots/nodules (Upreti and Murti, 1999a). Stoll *et al* (2000) showed that under partial root-drying there was reduction in cytokinin concentration, concomitant with an increase in the xylem sap pH in grapevine. Goiocchea *et al* (1995) reported a decrease in cytokinins in alfalfa under drought, and this was related with accelerated rate of senescence. In desert-grown almond trees, cytokinins showed peak concentrations in the morning and a rapid decrease in the afternoon; these fluctuations preceded daily variation in stomatal conductance (Fusseder *et al*, 1992).

The precise mechanism and cellular site of cytokinin action are not well understood. Brault and Maldiney (1999) propounded that cytokinins acted at the plasma membrane in association with other signaling molecules. In this context, cytokinins have been shown to antagonize many physiological processes mediated by ABA (Cowan *et al*, 1999). Some important processes induced by ABA and reversed by cytokinins are stomatal closure, leaf senescence and leaf expansion. This antagonism of ABA and cytokinins may be an outcome of metabolic interactions as cytokinins share a common biosynthetic origin with ABA. Cowan and Railton (1987) showed that a range of cytokinins reduced the incorporation of labeled mevalonic acid into ABA. Cytokinins have been shown to exert a response in stomata opposite to that of ABA. This increases stomatal aperture and transpiration in many plant species (Pospisilova *et al*, 2000).

Evidences showing cytokinins overriding the effects of ABA on stomata (Pospisilova *et al*, 2000, Blackman and Davies, 1985) revealed that reduction in cytokinins under stress would amplify shoot response to increasing concentrations of ABA. Davies (1995), thus, conceptualized that cytokinins act as the negative signal in plants undergoing drying. The mechanism of cytokinin action on guard cell involves direct action of membrane hyperpolarization by stimulation of adenylate cyclase activity that leads to increase in intracellular adenosine 3',5'-cyclic monophosphate content, stimulation of guanylate cyclase activity or interaction with a calcium calmodulin system (Pospisilova and Dodd, 2005, Incoll *et al*, 1990 Morsucci *et al*, 1991). The antagonism of cytokinins to ABA-induced stomatal closing may result from interactions in signal transduction pathway of both compounds, perhaps via the involvement of cytosolic calcium (Hare *et al*, 1997).

Stomatal opening is regulated by hydraulic as well as chemical signals, the relative importance of these signals being dependent on the growth-stage and growth-condition (Whitehead, 1998). Both naturally-occurring and synthetic cytokinins increase transpiration rate and increase stomatal aperture (Incoll *et al*, 1990, Incoll and Jewer, 1987). However, stomatal responses to cytokinins are found to be variable. Blackman and Davies (1983) revealed that Z alone did not affect stomatal opening, but partially reversed ABA-induced stomatal closure. In contrast, ZR or kinetin decreased stomatal opening and had no affect on ABA-induced stomatal closure.

Although cytokinin oxidase has been reported long back in the catabolism of cytokinins, little work has been carried out in relation to their involvement in stress tolerance. Manju *et al* (2001) revealed 3-fold increase in the activity of cytokinin oxidase in roots under stress and suggested it to be a regulatory enzyme of cytokinin level in roots of stressed plants.

c) Ethylene

Ethylene is the simplest olefinic gaseous hormone known to regulate a wide range of plant developmental processes. It is biosynthesized by conversion methionine to ethylene via the intermediates S-adenosyl methionine (SAM) and 1-amino cyclopropane-1-carboxylic acid (ACC) and enzymes ACC synthase and ACC oxidase (Fig. 5), (Yang and Hoffmann, 1984). Water stress is found to enhance ethylene level in French bean (Upreti *et al*, 1998, 2000), orange (Ben-Yehoshua and Aloni, 1974), avacado (Adato and Gazit, 1974), *Vicia faba* (El-Beltagy and Hall, 1974) and in many other plant species (Narayana *et al*, 1991, Guin, 1976, Irigoyen *et al*, 1992). The increase in ethylene under stress is of adaptive significance as it helps plants to cope with stress by reducing water-loss through increased senescence of fruits/leaves and reduced growth. The magnitude of ethylene changes under stress depend upon growth stage and stress duration (Upreti *et al*, 2000). The biochemical mechanism that provokes ethylene biosynthesis under stress is still not clearly understood and some reports also show variation in response. Naylor (1972)

suggested greater availability of methionine as a result of increased rate of protein breakdown under stress, leading to elevated levels of ethylene. Beltrano *et al* (1997) revealed that increased production of free radicals under water stress facilitated greater conversion of ACC to ethylene. Increase in ethylene level in response to stress is evident primarily by increased synthesis of ACC (Yang and Hoffmann, 1984). Xu and Qi (1993) reported that a slowly developing drought did not promote ethylene or altered ACC levels, while, rapidly developing drought enhanced both ethylene and ACC levels. Narayana *et al* (1991) also reported more ethylene upon rapid loss of water. Upreti *et al* (2000) found increase in ethylene under mild and moderate stress and decline in its concentration under severe stress regimes. Beltrano *et al* (1997) observed slight changes in ethylene in leaves under moderate or severe conditions. Wright (1980) and Hoffmann *et al* (1983) showed that ABA interacted with ethylene metabolism by regulating ACC levels. Also, ABA accumulation in sufficient quantity is found to be inhibitory to ethylene production (Spollen *et al*, 2000).

d) Polyamines

Polyamines are important growth regulatory polycationic molecules known to be involved in a wide range of developmental events including embryogenesis, root development and senescence (Galston and Kaur-Sawhney, 1990; Tiburcio *et al*, 1997) and also in plant responses to stress (Flores 1991; Galston *et al*, 1997; Kumar *et al*, 1997). In plants, polyamines are biosynthesized by decarboxylation of either ornithine or arginine in the reaction catalyzed by enzymes ornithine decarboxylase (ODC) and arginine decarboxylase (ADC) (Fig. 5) (Boucherneau *et al*, 1999).

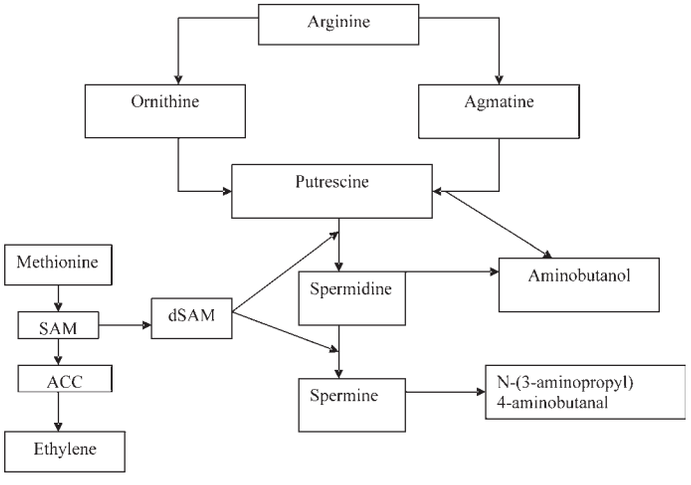

Fig 5. Biosynthesis of ethylene and polyamines (Liu *et al*, 2007)

This step leads to formation of putrescine, which in turn, by subsequent addition of aminopropyl moiety, produces spermidine (Spd) and spermine (Spm), respectively, in reactions catalyzed by Spd synthase and Spm synthase. The aminopropyl moiety results from decarboxylation of S-adenosylmethionine (SAM) by the enzyme SAM decarboxylase. The dynamics of polyamines metabolism are complex due to existence of degradation and conjugation pathways and of transport and uptake mechanisms (Martin-Tanguy, 2001, Federico and Angelini, 2001). Besides biophysical effect, through their positive charge at physiological pH, polyamines may be involved in signal transduction pathway, through effects on calcium fluxes (Thomas *et al*, 1993) and interaction with transcriptional factors (Wang *et al*, 1999) and protein kinases (Datta *et al*, 1987). Polyamines also interact with other growth regulators (Altaman, 1989). Polyamines and ethylene synthesis are linked through their common precursor, SAM. Several investigations have revealed that polyamines and ethylene inhibit each other's biosynthesis and action as a result of sharing a common intermediate (Tiburcio *et al*, 1997). Polyamines have also been shown to increase ABA in plants subjected to water stress (Upreti and Murti, 1999b).

Water stress leads to accumulation of free or conjugated polyamines in many plant species, indicating that polyamine biosynthesis play an important role in plant response to stress (Boucherneau *et al*, 1999, Liu *et al*, 2007). The increase in polyamines under stress may be a result of their *de novo* synthesis or reduced degradation (Alcazar *et al*, 2006a, Kao, 1997). However, the exact mechanism by which polyamine biosynthesis under stress are altered still remains to be elucidated. There are also some reports of decrease or no alteration in the levels of polyamines, thereby revealing competition in the mechanism of its biosynthesis under stress conditions. Differences in polyamine metabolism under stress depend upon plant species/cultivar, duration of stress, developmental stage, etc. (Liu *et al*, 2007). Upreti and Murti (2005) reported cultivar differences in polyamine changes in French bean under water stress (Fig 6). Moreover, the response of stress on an individual polyamine varied with duration of stress. Putrescine, which increased initially with stress, declined under severe stress regimes. In contrast, spermidine levels consistently declined and spermine levels progressively increased with stress. Spermine level under stress was related to ABA and to stress tolerance of the cultivar. Differential response of water stress to changes in individual polyamines is also shown by Turner and Stewart (1986).

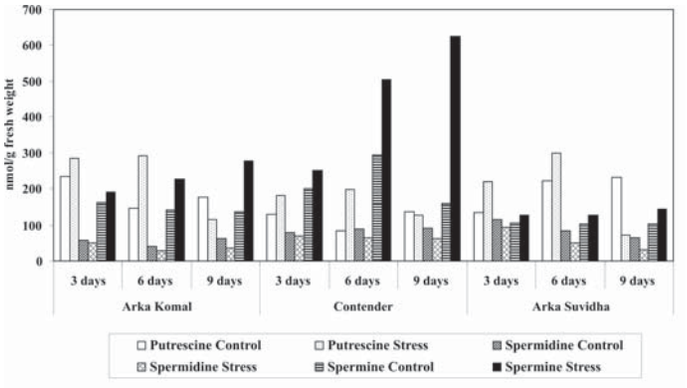

Fig 6. Free polyamines levels in French bean under water stress

Exogenous polyamine applications have been tried for providing evidence of its role in counteracting stress. Polyamine treatment increased endogenous polyamine levels in plants under stress (Tiburcio *et al*, 1997; Bagini and Torrigianni, 1992) and also reversed stress-induced changes in growth and cellular injuries. Stress-mitigating effects of individual polyamines, however, were different, because of differences in their absorption, transport and utilization among various plant species. Several lines of evidences have shown the positive function of polyamines in combating stress as being related to their antioxidative (Ormrod and Beckerson, 1986), free radical scavenging (Schuber, 1989; Malmberg *et al*, 1998), effects on ABA synthesis (Upreti and Murti, 1999b) and membrane stabilizing properties. Evidences provide a role of polyamine in modulation of stomatal aperture, an effect similar to that of ABA, possibly by targeting K^+ Arabidopsis Transporter (KAT1)-like inward K^+ channel in guard cells (Liu *et al*, 2000).

Investigations on gene expression associated with polyamines under drought have been made and reports indicate presence of a complicated transcriptional profiling (Gonzalez de Mejia *et al*, 2003). The mRNA of some polyamine biosynthetic genes is rapidly induced immediately after stress in some species and, in others; it is induced when stress is exerted for a certain period. This indicates that polyamine genes are differentially regulated under stress (Malmberg *et al*, 1998). The possible reason for differential expression of polyamines genes under stress is still unclear. Recent studies of Alcazer *et al* (2006b) depict up-regulation of polyamine biosynthetic genes by water stress as an ABA-dependent response.

e) Brassinosteroids

Brassinosteroids are naturally occurring compounds, well-documented for their role in plant growth and development (Clouse and Sasse, 1998). Their growth-regulatory activity is suggested to be a result of their influence on metabolic processes associated with photosynthesis, and nucleic acid and protein biosynthesis (Sasse, 1997). Brassinosteroids have also been shown to counteract stress effects in plants (Khripach *et al*, 2000). Brassinosteroid biosynthesis is divided into the sterol-specific pathway involving conversion of squaline to compesterol and brassinosteroid specific pathway involving compesterol to brassinosteroid (Agarwal and Gehlot, 2000). In brassinosteroid-specific pathway, compesterol undergoes a series of hydroxylation, reduction, epimerisation and oxidation reactions leading to formation of the oxidised form of brassinolide (Fujijoka and Sakurai, 1997; Choe *et al*, 1997). The last step in brassinosteroid synthesis is C-6 oxidation of castasterone. Brassinosteroids are reported to form 2, 3-glucosyl and acyl-conjugates at 3-position of its moiety (Fig. 7) (Abe *et al*, 1996).

Exogenous application of brassinosteroids is found to stimulate nucleic acid and protein synthesis and activates the ATP driven proton pump. There are also reports that

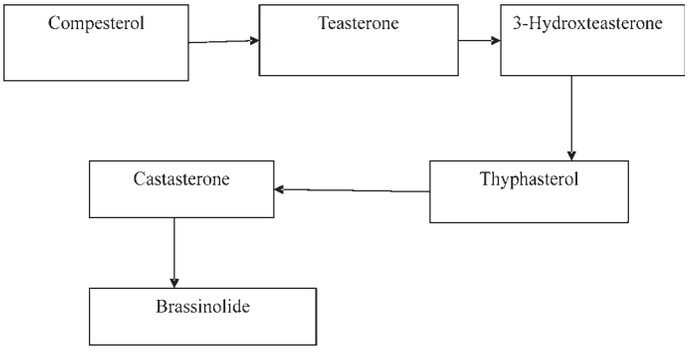

Fig 7. Possible biosynthetic pathway of brassinosteroid (Brosa, 1999)

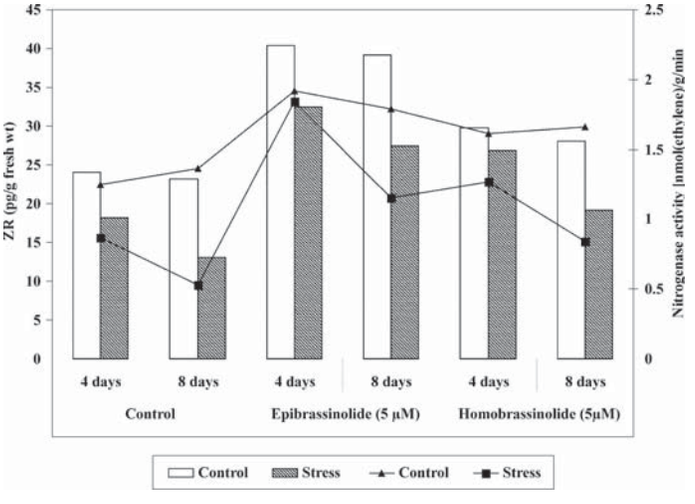

Fig 8. Effect of brassinosteroies on cytocinin-t-ZR content and nitrogenase activity in nodulated roots of French bean under water stress

these interact with other hormones such as auxins, ethylene and cytokinins in evoking physiological responses (Brosa, 1999). Information on changes in brassinosteroid content during water stress is lacking. Foliar application of the epibrassinolide is found to improve plant resistance to water stress by influencing nitrogenase activity and cytokinins levels in the roots (Fig 8.) (Upreti and Murti, 2004c). Xu *et al* (1994) reported a decline in stomatal opening and transpiration rate following brassinolide foliar application, and the treatment enhanced the effect of simultaneously-applied ABA. Rajasekharan and Blake (1999) found delay in stomatal closure induced by water stress following homobrassinolide treatment.

f) Plant growth regulators and stress amelioration in some horticultural crops

Besides conventional breeding and recent transgenic approaches, application of growth regulators in amelioration of water-stress tolerance has been attempted in a wide range of crops. In spite of a good number of studies, commercial success in growth regulator technology in combating stress effects is scant. This is because of the dependence of growth regulator action on factors such as crop species, cultivar, growth stage, stress severity, method of application, sensitivity of tissue, etc.

Potential of ABA as an anti-transpirant compound (to lower plant-water use) has been attempted in bell pepper while transplanting seedlings from greenhouse to the field or for hardening tissue-culture grown plants (Berkowitz and Rabin, 1988). Dipping of roots prior to transplanting exhibited greater survival of seedlings than those dipped in water. This facilitates the nursery industry to minimize maintenance-cost, extended marketing period and reduces the risk of dehydration during storage and transport. This effect of ABA, however, lasts for a short period due to faster breakdown of ABA in plants. To reduce ABA breakdown, Sharma *et al* (2005) employed ABA-analogs in tomato seedlings and found them to be effective in lowering plant water use for a longer period. The effectiveness of ABA-analogs, however, depended upon crop species, as these did not confer any positive effect in marigold (Sharma *et al*, 2005). Moreover, ABA treatment besides lowering transpiration also reduced photosynthesis rate in plants. But, Lovey (1984b) in his studies on grape stated that ABA effects on transpiration were much higher than on photosynthesis. Rajasekharan and Blake (1999) revealed that feeding of ABA through xylem, prior to imposing of water stress in *Pinus bankisana* improved tolerance by manipulating water use efficiency and reducing membrane damage. Pospisilova and Batkova (2004) found ABA treatment to be effective in ameliorating negative effects of water stress in French bean and sugar beet by improving plant water-balance through its effects on stomatal conductance and transpiration rate. Positive effects of ABA were also seen upon rewatering stressed plants.

Water-stress leads to a decline in cytokinin pool in the plants and, hence, the potential of benzyl adenine for mitigating stress response in plants was explored. Rulcova and Pospisilova (2001) witnessed a faster recovery of bean plants from water-stress following application of benzyl adenine. However, effects of the treatment broadly depended upon the concentration of benzyl adenine and were independent of method of application. Pospisilova and Batkova (2004) further observed that the role of benzyl adenine in lowering stress-effects was species-specific. Metwally *et al* (1997) found benzyl adenine and 4-CPPU to be effective in increasing the photochemical activity in stressed and rehydrated beans plants. Upreti and Murti (2000) reported that priming of French bean seeds with benzyl adenine improved seed-germination and seedling-growth under osmotic stress.

Triazole compounds such as cycocel and paclobutrazol have been shown to impart tolerance to water stress in many plant species (Fletcher *et al*, 2000). The precise mechanism by which these impose such effects is not very clear. One possibility is that this occurs through increased production of ABA by inhibiting gibberellin synthesis. When gibberellin synthesis is inhibited, more precursors in the terpenoid pathway accumulate and are diverted to ABA production. Increased ABA helps in plant water-balance, growth reduction and increased antioxidant content/activity (Davis and Curry, 1991). Sankhla *et al* (1989) found soil-drenching treatment with paclobutrazol as important in minimizing water-stress injuries in fruits of ber trees. Still and Pill (2004) found foliar application or seed-priming with paclobutrazol to improve water-stress tolerance in tomato seedlings, by increasing xylem pressure potential and lowering electrolyte-leakage and chlorophyll-loss. Swietlik and Miller (1983) observed an increased plant-water status in apple trees subjected to water stress. Similar effects with paclobutrazol are reported in strawberry (Navarro *et al*, 2007), peach (George and Nissen, 1992) and pea (Wang and Lin, 1992). Paclobutrazol is also found to improve resistance of micropropagated plantlets of chrysanthemum to desiccation (Roberts and Matthews,

1995). Paclobutrazol treatments are also found to induce morphological adaptation to water-stress in landscape plant *Phillyrea angustifolia*, allowing the plants to overcome transplant shock occurring later in transplanting. Paclobutrazol is also stated to improve water stress tolerance in many ornamental perennials and bedding plants (Channey, 2003). Prakash and Ramachandran (2000) reported cycocel as an effective anti-transpirant in brinjal grown under glasshouse conditions. Misra and Pradhan (1972) stated that cycocel and B-9 were effective anti-transpirants for tomato plants grown under water-deficit conditions. Upreti and Murti (2000) found that seed-priming with mepiquate chloride offered good germination in beans under osmotic stress.

Exogenous application of brassinosteroid has gained attention to modulate stress tolerance in the recent past. But there are only few reports that depict their successes in horticultural crops. Upreti and Murti (2004c) reported increase in pod yield in French bean under water stress following epibrassinolide treatment, by checking stress induced decline in root nodulation (Table 1).

SUMMARY

Endogenous growth regulators are vital components of plant growth and development under water stress conditions. Several reports have shown that water stress alters the level of growth regulators, and the resulting balance of growth regulators helps in providing better stress-adaptability to plants through their effect on stomatal functioning, plant water-balance and growth manipulation. There is either increase or decrease in the level of growth regulators in plants under stress. While stressed plants invariably show an increase in ABA and a decrease in cytokinin, the effects of stress on ethylene and polyamines in plants are variable.

Among growth regulators, researches on ABA have received wide attention in view of its involvement in stomatal functioning, osmotic adjustment, root to shoot signaling, gene expression and protein modification. Apoplastic ABA level that regulates stomatal aperture is controlled by synthesis, degradation, delivery and transportation of ABA within the plant. Other factors such as intercellular movement of calcium and potassium, together with pH and sugars, are vital in regulation of ABA-mediated stomatal closure. Water-stress alters protein synthesis and some of these proteins are also sensitive to ABA. The characteristic features of proteins help in establishing ABA-dependent stress perception-response pathway. However, information on ABA-specific proteins associated with stress-responses lacks clarity. Studies on ABA-sensitive and ABA-deficient mutants have indicated a role of ABA in stress adaptation mechanism in plants. At the molecular level, ABA-responsive genes have been identified and their expression has been characterized. Evidences show that some genes are up-regulated while others are down-regulated, resulting in net synthesis of the genomic product offering resistance against stress. However, information regarding ABA interaction with stress-responsive genes and the precise function of ABA-responsive genes still remains unidentified. Documentation of specific genes expression is important in gene-pyramiding associated with water-stress tolerance for developing superior tolerant genotypes. Significant cross-talk and interconnections are involved in stress-signaling. Systematic approaches with genomic analysis will help in resolving the complex network of signaling mechanism and elucidate the stress mechanism.

ABA-dependent signaling is also important in induction of antioxidant-defense response. An interaction between calcium and the reactive oxygen species is

Table 1. Effect of brassinosteroids on nodule number, mass of nodulated roots and pod-yield in French bean

Treatment	Conc. (µM)	Stress (d)	Nodule number		Nodulated root mass (g plant^{-1})		Pod-yield (g plant^{-1})	
			control	stressed	control	stressed	control	stressed
Control		4	39.3	23.0	1.57	1.20	119.7	79.3
		8	46.0	18.0	1.87	0.97	126.5	64.1
Epibrassinolide	1	4	54.0	33.7	2.07	1.60	126.7	95.3
	1	8	58.0	20.3	2.13	1.23	135.3	68.9
	5	4	72.3	50.3	2.90	2.43	157.6	128.7
	5	8	78.3	42.7	3.07	1.50	160.8	89.6
Homobrassinolide	1	4	43.0	29.0	1.90	1.63	126.7	85.4
	1	8	48.7	22.7	2.20	1.13	120.4	67.7
	5	4	64.0	45.3	2.60	2.00	145.8	111.5
	5	8	62.0	35.7	2.70	1.30	148.7	75.6

important in this respect. There are gaps as to how ABA regulates reactive oxygen species or how ABA-induced antioxidant defense is regulated. Answers to these will certainly strengthen knowledge on ABA involvement in stress-tolerance mechanisms in plants. Growth regulators, ABA, cytokinins, polyamines, etc., are synthesized both in leaves and roots of plants and are transported freely in the xylem sap and phloem, and partitioned in different tissues. ABA and cytokinins are interpreted as signals in stress-affected plants. Although there is a great deal of information on regulation of fluxes of these compounds in relation to water-stress, information on links between stress induced changes in soil conditions and generation of the signal is incomplete. There is also insufficient information on long-distance signaling via other chemicals, although some evidences of ABA interacting with ethylene and cytokinin have been provided.

Changes in polyamine content under water-stress are well-understood, but their functional significance in stress responses and defense needs to be elucidated. Brassinosteroids are well known for their functions at various physiological levels but their association in stress-mediation or stress-tolerance has not been fully explored. Exogenous application of growth regulators has been shown to accelerate the rate of plant acclimation to water stress. The effects of treatment, however, have been found to be dependent upon the species, cultivar, growth stage and stress-severity, because of which treatment responses are inconsistent. Systematic efforts are needed to further strengthen the scope of growth regulators in this area of research.

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Effects of growth regulators and explant-type on *agrobacterium*-mediated transformation in brinjal (*Solanum melongena* L.) cv. Manjarigota

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ABSTRACT

Effects of growth regulators and type of explants on transformation and *in vitro* morphogenetic responses of brinjal cv. Manjarigota were studied. Both hypocotyl and cotyledonary explants showed marked influence on *in vitro* morphogenetic responses after *Agrobacterium* co-cultivation. Hypocotyl explants showed callus initiation and regeneration responses earlier than cotyledonary leaves. Hypocotyl explants were found to be better than cotyledonary leaf explants in regenerating shoots after *Agrobacterium* co-cultivation. There was delay and reduction in both callus and regeneration responses in *Agrobacterium* co-cultivated explants. Hypocotyl explants showed the highest regeneration response on MS medium containing 2 µM BAP and 0.05 µM NAA while cotyledonary leaves did not show regeneration response after *Agrobacterium* co-cultivation. However, they showed green buds on MS medium containing 10 µM BAP and 1 µM NAA, which could not differentiate into shoots. Overall, hypocotyl explants were found better in regenerating shoots after *Agrobacterium* co-cultivation.

Key words: Growth regulators, explant, brinjal, transformation

INTRODUCTION

Agrobacterium-mediated transformation in plant species is the most widely used transfer system in plants which has been applied to transform and regenerate a few species with commonly used procedures (Van Wordragen and Dons, 1992). Brinjal is one of the crop plants in which in vitro plant regeneration was achieved on media supplemented with various growth regulators. (Sharma and Rajam, 1995; Gleddie *et al*, 1983; Magioli *et al*, 1998). The nature and concentration of growth regulators, in association with specific genotype, explant type and culture medium can cause significant differences in morphogenetic response of brinjal (Sharma and Rajam, 1995; Magioli *et al*, 1998; Magioli *et al*, 2000; Allichio *et al*, 1982; Gleddie *et al*., 1983). Usually, high-frequency regeneration protocols are employed in transformation studies. The adventitious shoot regeneration capacity of cells or tissues to be used in transformation studies significantly affects success in gene transformation (Yildiz *et al*, 2002). However, highly efficient protocols resulted in low transformation frequency and efficiency of less than 0.1 % in brinjal (Chen *et al*, 1995). Hence, it is necessary to analyze the effect of growth regulators and plant-related factors influencing *Agrobacterium* co-cultivation. 'Manjarigota' is the most preferred south Indian round type brinjal cultivar. Hence, we have made an attempt to study the effects of a basic operational step, growth regulators and explants on transformation and *in vitro* morphogenetic response in brinjal cv. Manjarigota during *Agrobacterium*-mediated transformation.

MATERIAL AND METHODS

Plant material

Genuine breeder-seed material of brinjal cv. Manjarigota was obtained from the Division of Vegetable Crops, IIHR. Seeds were soaked in gibberellic acid (100ppm) for three hours, dipped for 1 minute in 70 % ethanol, washed once in sterile distilled water, followed by sterilization for 8-10 minutes in sodium hypochlorite (approximately 4% available chlorine) solution and rinsed five times in sterile distilled water. These were germinated in culture tubes on half-strength MS (Murashige and Skoog, 1962) basal medium containing 3 % sucrose (w/v); pH was adjusted to 5.8 and the medium was gelled with 0.8 % agar. pH was adjusted to 5.8 before autoclaving.

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Media sterilization and culture conditions

Culture medium and instruments were sterilized by autoclaving at 121°C, 15 psi pressure for 20 minutes. Cultures were incubated in culture racks provided with white fluorescent tubes with a light intensity of 30-40µE m^{-2} s^{-1} under a 16 hr photoperiod in a culture room maintained at 25°C ± 2°C.

Explants

Fifteen to twenty day old-aseptically-grown seedlings, hypocotyl segments obtained after removing apical meristem and basal root stub (1cm long) and cotyledonary leaves separated from stalk and tip were used as explants.

Growth regulators

Hypocotyl explants were cultured on MS basal medium containing 3 % sucrose (w/v), 1, 2 or 3µM BAP 0.05 and 0.1 µM NAA and gelled with 0.8 % agar. Cotyledonary leaves were cultured on MS basal medium containing 3 per cent sucrose (w/v), 10 and 12.5 µM BAP in combination with 1, 2 or 3 µM NAA and gelled with 0.8 % agar.

Plant transformation

Agrobacterium strain A208 harboring the plasmid pBinBt-01 (Kumar *et al*, 1998) was used for plant transformation. The *npt*II gene conferring kanamycin resistance served as a selectable marker. Explants were precultured for two days on MS medium containing various hormones, depending on the explant-type. These were collected into a sterile petriplate, infected with *Agrobacterium* culture for 20-25 minutes, and placed back onto the parent medium. Explants were co-cultivated for two days, transferred onto culture media containing cefotaxime (500 mg/l) for two days and were then transferred onto medium containing cefotaxime (500 mg/l) and kanamycin (100 mg/l). Hypocotyl explants and cotyledonary leaf explants were cultured without *Agrobacterium* co-cultivation on MS medium containing hormones as specified for these explants, as control.

Data analysis

Observations on *Agrobacterium* overgrowth and health of explants were recorded every week for upto 4 weeks. Observations were further recorded after 4 weeks of culture on callus initiation and regeneration response. All treatments had six replications. Analysis of variance (ANOVA) was carried out to test statistical significance of the results observed. Fischers's Least Significant Difference (LSD) was used to determine statistical significance among means.

RESULTS AND DISCUSSION

Effect of growth regulators on transformation and morphogenetic response in brinjal cv. Manjarigota

In the present study, hypocotyl explants showed first signs of callus initiation and regeneration response at 8-10 days and 18 to 20 days of culture initiation, respectively. All the explants cultured showed callus initiation response. Growth regulator combinations significantly affected regeneration response in hypocotyl explants upon *Agrobacterium* co-cultivation (Table 1, Plate 1). Regeneration was highest (30.85 %) on hypocotyl explants grown in the presence of 2 mM BAP and 0.05 mM NAA, and lowest (18.27%) on 2 µM BAP and 0.1µM NAA. Inclusion of 0.05 mM NAA with BAP showed better regeneration response than 0.01 µM NAA.

Cotyledonary leaf explants showed the first signs of callus initiation and shoot bud initiation at 13-15 days, and four-six weeks of culture initiation, respectively. Cotyledonary leaf explants produced a profuse callus with adventitious roots. Highest number of explants showing green buds was recorded in cotyledonary leaf explants cultured on MS medium containing 10 mM BAP and 1 µM NAA (21%, with an average of 5.98 buds per explant) and

Table 1. Effect of Growth regulator concentration on transformation and morphogenetic response of hypocotyl explant in brinjal cv. Manjarigota

BAP µM	NAA µM response (%)	Callus initiation	Regeneration response (%)
1	0.05	100	25.55[ab]
1	0.1	100	21.31[ab]
2	0.05	100	30.85[a]
2	0.1	100	18.47[b]
3	0.05	100	26.96[ab]
3	0.1	100	18.27[b]

Fractions were converted into percentages; percentage data was subjected to angular transformation; CD= 11.74, SEm=2.060; differences are significant at 1 %; values followed by the same letter are not significantly different.

Table 2. Effect of growth regulators on transformation and morphogenetic response of cotyledonary leaf explant in brinjal cv. Manjarigota

BAP µM	NAA µM	Callus initiation (%) explant±SE	No. of green buds per buds (%) ±SE	Explants showing green
10	1	100	5.98±0.15	21 ± 3.41
10	3	100	3.46±0.20	13 ± 1.91
10	5	100	0.00±0.00	0 ± 0.00
12.5	1	100	4.71±0.16	17 ± 1.914
12.5	3	100	3.24±0.12	5 ± 1.000
12.5	5	100	0.00±0.00	0 ± 0.000

lowest from cotyledonary leaf explants cultured on MS medium containing 12.5 µ MBAP and 1 mM NAA (17 % of explants showed 4.71 green buds). Cotyledonary leaf explants did not show regeneration from shoot buds upon *Agrobacterium* co-cultivation (Table 2). Irrespective of the BAP level, explants cultured on MS medium containing 5 µM NAA did not respond.

Hormonal balance is a key factor in regulation of morphogenesis in cultured explants (Murashige, 1974). At similar ratios, varied concentration of cytokinin (BAP) and auxin (NAA) were used in earlier studies in hypocotyl explants (Matsuako and Hinata, 1979) and cotyledonary leaf explant culture of brinjal (Sharma and Rajam, 1995; Magioli *et al*, 1998) in regeneration studies. Addition of NAA at lower concentration resulted in increased shoot regeneration rate (Makay and Kitto, 1988) and presence of NAA was found to be necessary for *in vitro* regeneration in strawberry (Barcelo *et al*, 1998). Intrinsic hormone levels in a tissue make it respond better at a particular ratio and concentration of hormones, which depends upon the genotype and explant. Usually, high frequency *in vitro* regeneration protocol is used in transformation studies. No report is available on comparison of the effect of hormones on regeneration response in explants with and without *Agrobacterium* co-cultivation. However, various reports show that the effect of growth regulators on transformation frequency depends up on the cultivar and the explant in brinjal (Magioli *et al*, 2000; Billings *et al*, 1997).

Effect of explant on transformation and morphogenetic response in brinjal cv. Manjarigota

In the present study, callus initiation was not affected by hormonal combination upon *Agrobacterium* co-cultivation, both in hypocotyl and cotyledonary leaf explants. Similarly, *Agrobacterium* infection did not affect callus initiation response (96%) in hypocotyl explants. But, it had reduced callus initiation response in cotyledonary and leaf explants to the tune of 50-60 per cent in brinjal cv. Pusa Purple Long (Kumar and Rajam, 2005). However, it reduced callusing response in hypocotyl explants (Arpaia *et al*, 1997) and cotyledonary leaf explants (Prabhavathi *et al*, 2002) upon *Agrobacterium* co-cultivation in other, earlier studies. It appears that survival and response of explants in transformation varied due perhaps to the set of conditions employed in transformation protocol. In the present study, it is clear that *Agrobacterium* co-cultivation and the set of conditions during transformation were not detrimental to the explant and were optimum for survival and response of the explants. Hypocotyl explant is more sensitive to any type of treatment after excision (Yildiz *et al*, 2002), particularly, to *Agrobacterium* infection (Chakrabarty *et al*, 2002) compared to leaf explants (Arpaia *et al*, 1997). However, variation in response may be due to the crop, genotype, nature and physical status of explant along with the set of conditions used in transformation studies.

In the present study, delay in callus initiation and regeneration response was observed in both types of explants upon *Agrobacterium* co-cultivation and 7-8 day delay in callus initiation response was delayed by 5-6 days in hypocotyl explants and 7-8 days in cotyledonary explant, respectively, as compared to the control explant. 7-8 days delay in regeneration response and 2-3 weeks delay in appearance of green buds was observed in hypocotyl explants and cotyledonary explants, respectively compared to control explants. This delay may be due to the following reasons: 1) plant cells need to withstand the shock *Agrobacterium* infection 2) process of transformation has to occur and 3) only transformed cells show response on the selection medium and these have to multiply into sufficient numbers for expression of response. Similarly, callus initiation and regeneration response were delayed in explants co-cultivated with *Agrobacterium*, as compared to the control (without *Agrobacterium* co-cultivation) explant in brinjal (Billings *et al*, 1997).

There was a drastic reduction in the regeneration

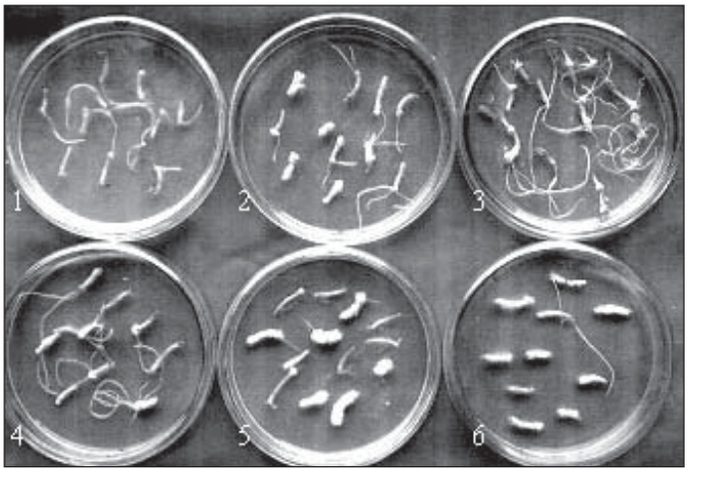

Fig 1. Regeneration response in hypocotyl explants upon *Agrobacterium* co-cultivation cultured on MS medium containing different hormone combinations: 1) 1 µM BAP+0.05 µMNAA, 2) 1 µM BAP+0.1 ìMNAA, 3) 2 µM BAP+0.05 µMNAA, 4) 2 µM BAP+0.1 µMNAA, 5) 3µM BAP+0.05 ìMNAA, 6) 3 µM BAP+0.1 µMNAA

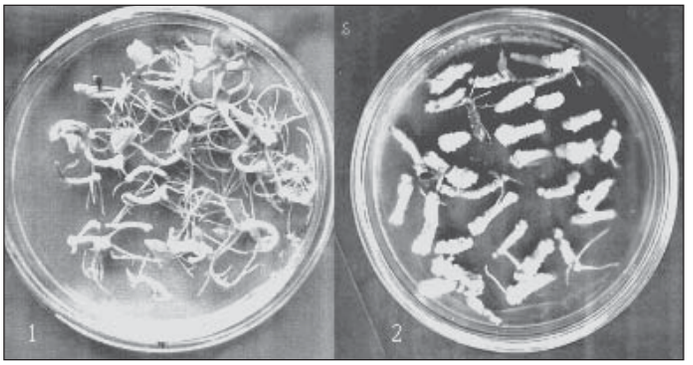

Fig 2. Comparison of regeneration response in hypocotyl explants (1) without and (2) with *Agrobacterium* co-cultivation

response of hypocotyl explants and a complete lack of response of cotyledonary leaf explants upon *Agrobacterium* co-cultivation as compared to the control explants (Plate. 2). Cotyledonary leaf explants produced green buds which could not differentiate into shoots. The occurrence of delayed and reduced regeneration response from explants upon *Agrobacterium* co-cultivation is not uncommon. Possible explanations for this phenomenon are: 1) plant cells may perceive *Agrobacterium* infection as an attack and 2) the inoculation process may influence plant regeneration negatively. Similarly, shoot bud differentiation was drastically reduced in explants subjected to *Agrobacterium* infection in cauliflower (Chakrabarty *et al*, 2002). Hypocotyl explants were most responsive upon *Agrobacterium* infection. Furthermore, early colonization of *Agrobacterium* was a major problem with cotyledonary leaf explants. It might be due to uneven surface of leaf explant, which was not completely exposed to culture media containing cefotaxime.

Arpaia *et al* (1997) reported reduced callusing response in both hypocotyl and cotyledonary leaf explants upon *Agrobacterium* co-cultivation. However, higher regeneration response was noticed in kanamycin-resistant calli obtained from hypocotyl explant as compared to that from cotyledonary explant. Kumar and Rajam (2005) reported higher callus initiation response and lower regeneration response from hypocotyl explant compared to cotyledonary leaf and leaf explants. Therefore, experimental conditions other than type of explant may be responsible for differences in response during transformation. Hypocotyl explant showed better regeneration response upon *Agrobacterium* co-cultivation in brinjal cv. Manjarigota than did cotyledonary leaf explant. Similarly, hypocotyl explant resulted in the highest transformation efficiency compared to leaf and cotyledonary leaf explants in perilla (Lee *et al*, 2005). Hypocotyl explants were successfully used in *Agrobacterium*-mediated transformation in chilli (Nianiou *et al*, 2000) and cauliflower (Chakrabarty *et al*, 2002). In conclusion, it is stated that hypocotyl explant is better as compared to cotyledonary leaf or leaf tissue for transformation studies in brinjal. The present study also vindicate that morphogenetic response varies with growth regulator and explant type in brinjal.

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A revised protocol for *in vitro* propagation of *Carica papaya* using lateral buds from field-grown trees

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ABSTRACT

A revised protocol has been developed for *in vitro* propogation of papaya using explants from field-grown trees. Successful establishment of papaya *in vitro* using lateral buds could be obtained by treating the buds with Carbendazim (0.2%) and Streptomycin (0.1%) for 24h, followed by surface sterilization with mercuric chloride (0.1%) for 3 minutes and culturing on MS medium supplemented with BAP (0.3 mg/l) and NAA (0.1 mg/l). Established buds were proliferated on modified MS medium supplemented with BAP (0.3 mg/l) and NAA (0.1 mg/l). Modified MS medium supplemented with BAP (0.3 mg/l), NAA (0.1 mg/l) and GA_3 (1 mg/l) caused extensive elongation of shoots. Elongated shootlets were rooted on half-strength MS medium supplemented with BAP (0.1mg/l), NAA (0.1 mg/l) and IBA (2 mg/l). Rooted plantlets were initially hardened on a potting mixture consisting of soilrite and later on a mixture of sand, soil and FYM (1:1:1).

Key words: Micropropagation, mature explants, *Carica papaya*

INTRODUCTION

Papaya, being a highly cross-pollinated crop, is polygamous in nature when propagated through seeds. It is cultivated worldwide using dioecious cultivars in the sub-tropical region and with gynodioecious cultivars in the tropical region, which segregate into female and hermaphrodite offspring. In commercial cultivation, one third of the females in a gynodioecious population need to be removed as these have limited economic value. Dioecious varieties normally produce 50% male plants, if propagated by seed. In addition, the papaya ring spot virus (PSRV) is a major disease in papaya causing 70-80% loss in plantations. Though this can be overcome using resistant varieties, these would lose their resistance if propagated by seeds. These problems however, can be solved if the plants are clonally propagated.

Clonal propagation through *in vitro* methods of known sex types is a better option since conventional techniques like use of cuttings and grafting have resulted in limited success. Papaya, being polygamous, requires that the explants be excised from a known sex type, which can be realised only when the tree attains reproductive maturity. Thus sex determination in papaya plants at the seedling stage or selecting explants from the mature tree enables propagation of the known sex. Successful true-to-type propagation under *in vitro* conditions can be achieved if explants are taken from mature, field-grown trees. Studies on use of lateral buds from field-grown trees have been successful under *in vitro* conditions but commercial exploitation on large scale remains unexploited due to lack of a micropropagation protocol. Hence, clonal propagation of individuals of known sex can be successfully applied to true-to-type propagation of *Carica papaya*.

MATERIAL AND METHODS

Explant preparation

Axillary buds were dissected from nodes of field-grown hermaphrodite, bearing plants of var. Surya in plastic covers and kept under running water with 1-2 drops of Tween-20 for 2h to minimize the flow of latex. These explants of 4-5mm size were pre-treated with carbendazim (0.2%) and Streptomycin (0.1%) for 24h on a shaker at 150 rpm, followed by surface-sterilization with mercuric chloride (0.1%) for 3 min. The explants were rinsed 4-5 times in sterile distilled water to wash off residual sterilants and were then inoculated on Medium. In all the experiments 20 explants were taken and replicated three times.

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Media and culture conditions

Explant establishment

Treated explants were inoculated onto Murashige and Skoog (1962) basal medium supplemented with different concentrations and combinations of cytokinins viz., BAP (0.2, 0.3, 0.5, 2.5 mg/l) and kinetin (2.5mg/l) and auxins NAA (0.1, 0.2, 0.5 mg/l), IAA (0.175, 0.2, 0.5, 1.0 mg/l). Media were gelled with 0.8% agar. pH of the media was adjusted to 5.8 prior to autoclaving at 103.4 kPa for 20 min.

Subculture for proliferation and elongation

Contamination-free cultures were sub-cultured onto establishment medium at every 15 days. The establishment medium comprised of Murashige and Skoog (1962) basal medium supplemented with various concentrations and combinations of plant growth regulators (NAA at 0.1, 0.2 and 0.5mg/l, IAA at 0.175, 0.2, 0.5 and 1.0mg/l, BAP at 0.2, 0.3, 0.5 and 2.5mg/l and Kinetin at 2.5mg/l). The same medium was used for proliferation of explants.

When the shootlets were nearly 2mm in length, they were transferred to elongation medium containing MS basal salts with BAP (0.3mg/l), NAA (0.1mg/l) and Gibberellic acid (GA_3) (0.5, 1.0 and 2.0mg/l).

Subculture for rooting

Well-developed shoots (3-4 cm long) were then transferred onto rooting medium to induce rhizogenesis under *in vitro* conditions. To promote *in vitro* rhizogenesis, ¾, ½, and full strength Murashige and Skoog (1962) basal medium supplemented with different concentrations and combinations of plant growth regulators (IBA at 0.5, 1.0 and 2.0mg/1, NAA at 0.1mg/l and BAP at 0.1mg/l) were used.

Acclimatization

Well-developed shootlets of *Carica papaya* with *in vitro*-formed roots were removed from culture media and transplanted into netted pots containing Soilrite™. These were maintained at 90% relative humidity by covering with polythene. Later, holes were punched on these covers to permit transpiration. During the hardening period, temperature of 25±1°C and 16h photoperiod was maintained. The *in vitro* hardened *Carica papaya* plantlets were further hardened under *ex vitro* conditions with sterilised FYM: sand: soil mixture in the ratio of 1:1:1. Subsequently, these primary hardened plants were transferred (at 1½ months) to greenhouse conditions and maintained there for further field-planting.

Culture incubation

Cultures were incubated at 16h photoperiod, at 25±1°C under white cool fluorescent light having an intensity of 30limol/m^2/sec.

RESULTS AND DISCUSSION

Effect of 6-benzyl amino purine on shoot proliferation

In the present investigation (Table 1), explants were cultured on MS basal medium supplemented with NAA at 0.1 mg/l and different concentrations of BAP (0.1, 0.2 and 0.5 mg/l). Inclusion of BAP at 0.3 mg/l recorded the highest proliferation of 71 and 85% at 7 and 15 DAI, respectively, with low callusing. Higher concentration of BAP (0.5 mg/l) recorded a proliferation of 71% both at 7 and 15 DAI, with moderate callusing at the base of the explants. These results are contrary to the findings of Litz and Conover (1978),

Table 1. Effect of different concentrations of BAP on proliferation of papaya cultures

Type of proliferation at 7 DAI**			
Proliferation category*	BAP at 0.2 mg/l	BAP at 0.3 mg/l	BAP at 0.5 mg/l
VGP	14%	7%	0%
GP	0%	35%	7%
P	22%	43%	64%
NP	64%	7%	14%
NR	Nil	8%	15%
Type of proliferation at 15 DAI			
	BAP at 0.2 mg/l	BAP at 0.3 mg/l	BAP at 0.5 mg/l
VGP	14%	14%	0%
GP	7%	29%	7%
P	22%	36%	64%
NP	29%	7%	14%
NR	28%	14%	15%

* VGP- Very good proliferation, GP- Good proliferation, P- Proliferation, NP- No proliferation, NR-No response
** DAI- Days After Inoculation

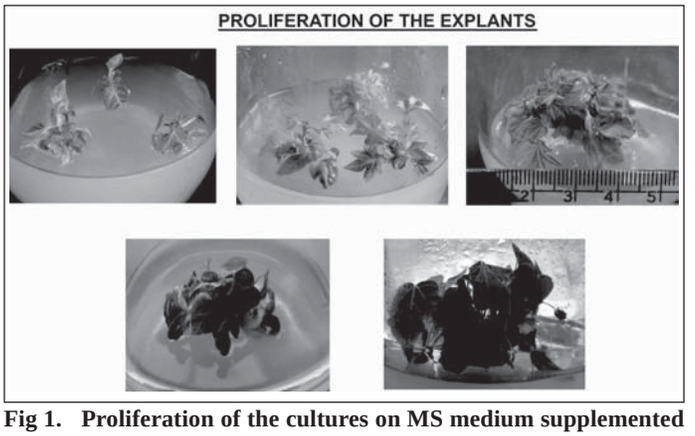

Fig 1. Proliferation of the cultures on MS medium supplemented with BAP(0.3mg/l) and NAA (0.1mg/l)

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Reuveni *et al* (1990) and Drew (1988) who recorded higher multiplication rate with lowest callus production on Murashige and Skoog (1962) medium supplemented with BAP at 0.5mg/l and NAA at 0.1mg/l. In the present study, an average of five-fold increase (Fig 1) was observed upto 10 subcultures and this remained static thereafter, which is in accordance with the findings of De Winnaar (1988) who obtained a 7-fold increase in each subculture until eight cycles and then became static. Litz and Conover (1978) too observed a 7-fold increase in plant number during every cycle and cultures continued to proliferate even after the 8th subculture. Varied response of explants, in the present study, to multiple shoot proliferation may be due to the plant species, clone, physiological state of the explants, endogenous, status of cytokinins and source of the chemicals.

Effect of GA_3 on shoot elongation at different intervals

GA_3 is known to cause elongation of shoots when applied as a supplement in the medium. In the present study (Table 2), explants were cultured on MS basal medium supplemented with BAP at 0.3 mg/l, NAA at 0.1 mg/l and varying concentrations of GA_3 (0.5, 1.0 and 1.5 mg/l) for elongation of the shootlets. Tufts of the proliferated, multiple shoots were transferred onto the elongation medium after observing maximum proliferation. Results revealed that inclusion of GA_3 at 0.5 mg/l and 1 mg/l gave maximum elongation of shoots (shoot length of 2 cm) (Fig 2). De Winnaar (1988) used GA_3 in the proliferating medium which induced shoot elongation although it reduced the multiplication rate. Results in the present study are similar to the findings of De Winnaar (1988) wherein multiplication rate was lower on elongation medium compared to that in proliferation medium (Table 3). Results obtained by Reuveni *et al* (1990) are contrary to the present research findings wherein GA_3 did not have any significant effect when used for elongation of shootlets. Elongation of shootlets was also observed after prolonged culture in rooting media in papaya (Siddique *et al*, 1999).

Table 2. Effect of different concentrations of GA3 on shoot elongation in papaya shoot buds under in vitro conditions

GA3 concentration (mg/l)	VLE	E	NE	NR
Shoot elongation* at 15DAI**				
0.5	36%	14%	50%	-
1.0	36%	50%	14%	-
2.0	43%	14%	36%	7%
Shoot elongation at 30DAI				
0.5	35%	21%	43%	-
1.0	29%	64%	7%	-
2.0	21%	50%	29%	-

* E – Elongated, VLE – Very little elongation

**DAI- Days After Inoculation

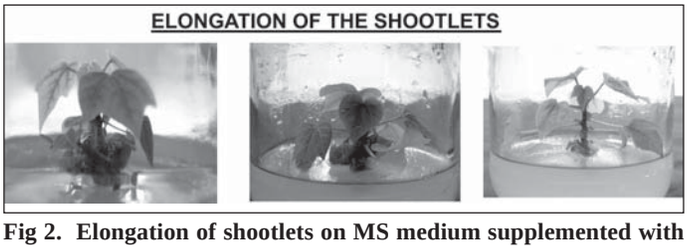

Fig 2. Elongation of shootlets on MS medium supplemented with BAP(0.3mg/l),NAA(0.1mg/l) and GA3(1mg/l)

Effect of basal medium on per cent root induction

A reduced mineral concentration in the medium increases the root initiation as reported by Drew (1987). In

Table 3. Mean multiplication rate per subculture of papaya shoots at different stages of subculture

Stage of subculture	Multiplication rate per culture cycle	
1	4.02	Subcultured on proliferation
2	5.26	medium without GA_3
3	6.69	
4	7.02	
5	4.27	Subcultured on proliferation
6	4.47	medium containing GA_3
7	5.23	
8	6.14	
9	6.32	
10	6.26	
Mean	5.57	
SEm±	0.176	
CD *(P=0.01)*	0.659	

Table 4. Effect of strength of basal medium on root initiation

Treatment	Per cent root induction	Mean number of roots per shoot	Mean root length of roots (cm)	Mean number of secondary roots (scoring)
MS	20	1.990	4.316	2.160
½ MS	45	3.800	3.075	4.710
¾ MS	37	1.740	2.699	2.030
S Em±		0.134	0.117	0.124
CD *(P=0.05)*		0.391	0.341	0.362
CD *(P=0.05)*		0.528	0.461	0.489

Number of replications per treatment = 10

Number of secondary roots	Scoring
0	0
1-5	1
6-10	2
11-15	3
16-20	4
21-25	5
26-30	6

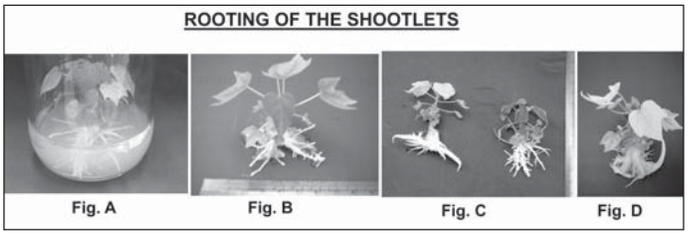

Fig 3. A-D: Rooting of the shootlets A-B: Rooting of the shootlets on ½ MS Supplemented with BAP(0.1mg/l), NAA(0.1mg/l) and IBA (2mg/l). C-D: Nature of roots grown on ½ MS Supplemented different concentrations of IBA

the present study (Table 4) different levels of Murashige and Skoog basal medium viz., full MS, ½ MS, ¾ MS were tried along with BAP at 0.1mg/l and NAA at 0.1mg/l. Culturing on ½ MS proved to induce higher percentage of root induction (45%) compared to ¾ MS (37%) and full MS (20%)(Fig 3).

The results are contrary to the findings of Teo and Chan (1994) who obtained 33% of rooting on MS medium and 26% of rooting on ½ strength MS indicating lesser percentage of root induction on reduced mineral salts (½ MS) than the normal medium (MS). Results of the present investigation revealed that reduced mineral concentration increased root initiation (45%) as against 20% on full MS thus indicating the favourable influence of reduced salt concentration on root induction. Bonga (1982) also reported that, reduction in mineral concentration has the influence on root number and initiation with tissue culture of tree species. However, Drew (1987, 1988) obtained only 30% rooting of shoots derived from mature tissue while 90% of those from 6-month-old plants within 3 weeks indicating the influence of explant age on rooting. Drew (1987) reported maximum rooting (68%) on cultures with only distilled water with 1% agar.

Effect of different concentrations of IBA on rooting

Experiments involving IBA using ½ MS basal along with BAP at 0.1mg/l and NAA at 0.1mg/l supplemented with different concentrations of IBA (0.5, 1.0 and 2.0mg/l) were tried to increase the rooting efficiency (Table 5). Best rooting (48%) of cultured shoots was achieved with ½ strength MS supplemented with BAP at 0.1mg/l, NAA at 0.1mg/l and IBA at 2mg/l. Plants on this treatment initiated more roots per plants and had better quality root system than those on IBA treatment at 0.5mg/l or 1.0 mg/l (Fig.3). Drew (1987) reported that using IBA at 2mg/l in the medium promoted good rooting of shoots in papaya. Higher concentrations of IBA and NAA in the medium caused abnormal root formation. Difference in quality of root system on plants grown on IBA and NAA

Table 5. Effect of different concentration of IBA on rooting

Treatment (I BA)	Per cent rooting	Mean number of roots per shoot	Mean root length (cm)	Mean number of secondary roots (scoring)	Nature of the root
0.5 mg/l	23	0.640	1.085	1.270	Thick blunted root
1.0mg/l	35	1.290	1.657	1.410	Thick long root with less number of secondary roots
2.0 mg/l	48	3.380	3.351	3.890	Thin, long and higher number of secondary roots
SEm±		0.133	0.113	0.126	
CD *(P=0.05)*		0.386	0.329	0.366	
CD *(P=0.01)*		0.521	0.445	0.494	

Number of replications per treatment = 10

has been also observed in grapevine and camellias (Novak and Juvova, 1982; Samautin *et al*, 1986).

In the present study, 48 % rooting was observed in plantlets Drew (1988) reported 90% rooting. However, the reason for low percentage of rooting may be light intensity, maintained at 130µ mol/m^2/sec with 16h photoperiod throughout the growth period which recorded an inhibitory effect on root induction. Drew (1987) reported the use of 80µ mol/m^2/sec during the root induction. However after the root initiation the growth increased as the light on the foliage increased (Drew, 1987).

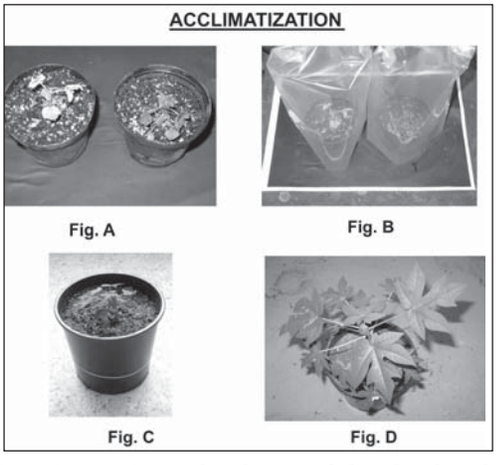

Fig 4. A-D: A-B: Primary hardening of the plantlets developed *in vitro*. C-D: Secondary hardening of the plants developed *in vitro*

Acclimatization

Acclimatization of well-developed plantlets of *Carica papaya* with *in vitro* formed shoots and roots was achieved on transplantation into netted pots containing soilrite (Fig 4). These plantlets were hardened under *ex vitro* conditions with sterilized FYM: sand: soil mixture in the ratio of 1:1:1. Later, these primary hardened plants were transferred (at 1 ½ months) to greenhouse conditions (Fig 4). Success rate for acclimatization during this stage was 90% and when plantlets were transferred to the field, all were established (Fig 5). These plants grew well and

Fig. 5 : Tissue cultured plants flowering in the field

produced fruits. Similarly, Hari Prakash *et al* (1996) could harden *in vitro* generated plantlets of guava in the same combination of potting mixture (sand: soil: FYM) in the ratio of 1:1:1. Hazarika *et al* (1998) could harden *in vitro* developed plantlets of citrus by loosening the caps after 4-6 weeks of rooting. Later, these primary hardened plantlets were transplanted into a mist-house indicating the need of the plantlets for gradual change in relative humidity and temperature during acclimatization, in the present investigation.

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Breeding French bean (*Phaseolus vulgaris* L.) for resistance to rust (*Uromyces phaseoli* Reben Wint.)

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ABSTRACT

French bean is an important legume vegetable grown for its tender, green pods for both fresh consumption and processing. Rust, caused by *Uromyces phaseoli,* limits successful cultivation of this crop. Popular varieties like Contender, Pant Anupama, Pusa Parvathi, Arka Komal, Arka Suvidha, etc., are susceptible to this disease. The french bean variety, Arka Bold, having resistance to rust was used in hybridization with Arka Komal, a popular bush variety with high yield and slender, long green pods but susceptible to rust. Inheritance studies indicated that resistance to rust was controlled by a single, dominant gene. Pedigree method of breeding was followed for incorporating rust resistance in to commercially cultivated varieties. Breeding lines with resistance to rust were selected to F_2 generation onwards. These were advanced up to F_7, wherein, a promising line, (Arka Bold x Arka Komal) 99-17-2-1-4-12-3, with resistance to rust with high pod yield and good pod quality was selected and named Arka Anoop and released for commercial cultivation.

Keywords: French bean, rust resistance,

INTRODUCTION

French bean (*Phaseolus vulgaris* L.) is one of the most important legume vegetables grown for its tender green pods. Globally it is grown in an area of 0.68 million ha with total production of 4.7 million metric tonnes and productivity is 6.91 tonnes / ha. In India, it is grown in an area of 0.15 million ha with annual production of 0.42 million metric tonnes (Anonymous, 2006). The crop is susceptible to various biotic and abiotic stresses. Among the various biotic stresses, rust caused by *Uromyces phaseoli* (Reben Wint) has become endemic in bean producing areas. The yield loss due to this disease is 78 to 90 % (Grafton *et al,* 1985) and it is serious during *rabi*. The disease incidence will be less during *kharif* season. The disease is more severe in tropics than in the temperate region and the pathogen will be more active under moderate temperature of 17 to 27^0 C and relative humidity of more than 95 %. The popular varieties like Contender, Pant Anupama, Pusa Parvathi, Arka Komal, Arka Suvidha etc., are susceptible to rust disease. Although chemical control using sulphur fungicides and propioconozole are recommended, the induction of genetic resistance will have the greater merit over the chemical control. Hence, the present study was taken up with the objective of developing a french bean variety with resistance to rust disease along with high yield and good pod quality and also to study the genetics of disease resistance.

MATERIAL AND METHODS

The source of resistance to rust was found in Arka Bold (Mohan *et al,* 1997). The hybridization was done between Arka Bold and Arka Komal (a bushy variety with high yield and slender long pods) in both combinations during 1999 at IIHR, Bangalore. Subsequently, F_1, F_2, B_1 and B_2 populations were raised and evaluated for resistance to rust. Artificial screening for rust was done by spraying uredospore suspension uniformly on both sides of the leaves. The concentration of spore suspension was maintained at 10^7 spores / ml. Percent disease index (PDI) was calculated as per the method given by Stavely (1983). The disease scoring was done on a 0- 9 scale where 0 = no pustules; 1 = small brown pustules covering less than 1% of leaf area; 3 = typical pustules covering 1-10 % of leaf area; 5= typical pustules covering 11- 25 % of leaf area; 7= typical pustules covering

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26 - 50 % of leaf area and 9= typical pustules covering more than 51 % of leaf area combined with withering of leaves. Per cent disease index (PDI) was calculated by using the formula given by Wheeler (1969),

$$PDI = \frac{0\ (\chi_0)+1\ (\chi_1)+3\ (\chi_3)+5\ (\chi_5)..... \times 100}{\chi_0+\chi_1+\chi_3+\chi_5....+\chi_n \times \max.scale\ (9)}$$

Where χ represents the diseased leaves within the sample plants in the respective class such as 0, 1, 3, …9. Data obtained from the two crosses and two testcrosses were subjected to χ^2 analysis. Based on the disease reaction in F_1, F_2, B_1 and B_2 population, the inheritance of resistance to rust was worked out. The plants with PDI less than 5 % were considered as resistant and those showing PDI more than 5 % as susceptible for formation of classes for test. Data of two crosses and two testcrosses were subjected to analysis to test the goodness of fit against assumed phenotypic ratio of 3:1(resistant and susceptible respectively) for single dominant gene controlling rust resistance. Pedigree method of selection was followed up to F_7 generation. Replicated yield trials were conducted for selected breeding lines.

RESULTS AND DISCUSSION

All the F_1 plants in the cross Arka Bold (R) and Arka Komal (S) and its reciprocal were resistant indicating the dominance over susceptibility and had shown that cytoplasmic genes are not involved in resistance. In F_2 population of 196 plants, observed segregation ratio for resistance to susceptibility, was 146: 50 as against expected ratio of 147:49 (Table 1). In the reciprocal cross, 216 plants were resistant and 68 were susceptible as against expected ratio of 213:71 respectively. Further, the test cross progeny of Arka Bold (R) and Arka Komal (S) segregated in the ratio of 55 resistant to 50 susceptible plants as against the expected ratio of 53: 53 respectively out of 106 test cross plants. In another test cross progeny of Arka Komal (S) and Arka Bold (R), the ratio was 54:48 as against 51:51. The calculated χ^2 value for both the F_2 and testcrosses were non significant with high probability of 0.87 to 0.89 and 0.55 to 0.63, respectively. F_2 population of both the crosses showed a good fit of 3:1 between resistant and susceptible and test cross progeny indicated the segregation in 1:1 ratio (Table 2). The cross, Arka Bold x Arka Komal indicated dominance of resistance to rust over susceptibility in F_1 progeny. This was similar in the reciprocal cross also. The pattern of segregation in F_2 population along with the two test cross generations followed Mendelian ratio of dominance and application of χ^2 test for F_2 and test cross generations indicated that resistance to rust was inherited as a single dominant gene in french bean variety, Arka Bold. These findings are in conformity with Augustin *et al*, (1972a, b), Stavely (1984), Stavely and Grafton (1985), Grafton *et al*, (1985), Finke *et al*, (1986), Sayler *et al*, (1995) and Yuebin (1995) who reported that the resistance to rust is monogenically controlled.

Further, Pedigree method of breeding was followed and segregants with resistance to rust were selected from F_2 generation onwards and were advanced up to F_7 generation, wherein, a promising line (Arka Bold x Arka Komal)-99-17-2-1-4-12-3 possessing resistance to rust with high yield and good pod quality was selected and named as

Table 1. Frequency of resistant and susceptible plants in parents and F_1's

Sl No.	Crosses	First parent		Second parent		F_1		F_2		Test Cross		BC with R Parent.	
		R	S	R	S	R	S	R	S	R	S	R	S
1	B x A	87	0	0	89	78	0	147	49	55	50	91	0
2	A x B	0	89	87	0	85	0	216	68	54	48	105	0

R=Resistant, S=Susceptible, A=Arka Komal, B=Arka Bold, BC=Back cross.

Table 2. Frequencies of F_2 and test cross progenies with their χ^2 estimates

Sl No	Crosses	Observed ratio		Expected ratio		Total	Chi Square	Probability
		R	S	R	S			
1	B x A	146	50	147	49	196	0.03	0.87
2	A x B	216	68	213	71	284	0.02	0.89
	Pooled	350	120	360	110	470	0.18	0.68
	Test crosses							
1	(B x A) x A	55	51	53	53	106	0.24	0.63
2	(A x B) x A	54	48	51	51	102	0.35	0.55
	Pooled	109	99	104	104	208	0.96	0.33
	Table χ^2 @ 1df.						3.84	

R=Resistant, S=Susceptible, C=Contender, A=Arka Komal, B=Arka Bold, K=KPV-1, BC=Back cross.

Fig 1. Arka Anoop a new french bean variety

Fig 2. French bean var. Arka Anoop (on either side) showing resistance to rust with susceptible check in the middle

Arka Anoop (Fig 1 and 2). Replicated yield trials conducted at IIHR, Hessaraghatta for three years from 2003 to 2005) during *rabi* season showed that the new variety, Arka Anoop had a significantly higher number of pods per plant (42.50) as compared to check (Table 3). It also recorded an average pod yield of 19.78 t /ha, as against a yield of 14.29 and 8.24 t/ha in the check varieties Arka Komal and Contender, respectively. The percent yield increase in Arka Anoop over check varieties Arka Komal and Contender was 38.42 and 140.05 respectively. Arka Anoop was completely resistant

Table 3. Average plant and pod characters of french bean var. Arka Anoop compared with parents and checks

Sl. No.	Characters	Arka Anoop	Arka Komal	Arka Bold	Contender	*P=0.05*	CV %
1	Days to 50 % flowering	32.50	32.00	33.0	32.50	1.41	2.05
2	Days to pod maturity	45.00	45.50	47.0	43.50	1.45	1.97
3	Plant height	58.50	57.50	55.0	52.50	2.33	4.72
4	Pod length (cm)	17.60	15.75	14.5	14.25	1.03	2.93
5	Pod width (cm)	1.00	1.05	1.55	1.00	0.11	4.24
6	Number of pods per plant	42.50	31.58	22.5	15.50	2.35	6.98
7	Ten pod weight (g)	88.50	56.00	75.0	51.00	11.00	5.79

Table 4. Average pod yield (t/ha) and rust index (PDI) of french bean var. Arka Anoop between 2003-2005 during *rabi*

Sl. No.	Varieties	Pod yield (t/ha)			Average	Rust PDI			Average	% Yield increase over Arka Komal	% Yield increase over Contender
		2003	**2004**	**2005**		**2003**	**2004**	**2005**			
1	Arka Bold (res. parent)	14.50	15.20	14.80	14.83	2.15	1.86	2.64	2.22	-	-
2	Arka Komal (Susc. Parent)	17.11	16.10	9.67	14.29	27.90	36.45	56.84	40.40	-	-
3	Arka Anoop	18.71	19.38	21.24	19.78	2.35	1.79	1.58	1.91	38.42	140.05
4	Contender (susc. check)	9.92	8.70	6.10	8.24	45.36	51.30	74.36	57.01	-	-
	CD *(P=0.05)*	2.43	1.92	1.76	-	5.79	2.83	4.85	-	-	-
	CV %	14.04	5.92	5.20	-	7.66	11.26	9.53	-	-	-

Table 5. Average pod yield (t/ha) of Arka Anoop during *Kharif* season

Sl. No.	Varieties	2003	2004	2005	Average	% increase over Arka Komal	increaseover % Contender
1	Arka Bold (res. parent)	15.50	14.30	14.90	14.90	-	-
2	Arka Komal (Susc. Parent)	18.00	18.50	17.22	17.90	-	-
3	Arka Anoop	18.83	21.13	19.90	19.95	15.85	69.00
4	Contender (susc. check)	10.50	12.25	12.69	11.81	-	-
	CD*(P=0.05)*	0.71	2.82	3.59	-	-	-
	CV %	4.22	7.85	15.00	-	-	-

to rust with very low PDI of 1.91 whereas, the check varieties, Arka Komal and Contender were susceptible (Table 4). Yield trials were also conducted during *kharif* seasons for three years from 2003 to 2005 wherein, Arka Anoop gave an average green pod yield of 19.95 t /ha, while in parents Arka Bold and Arka Komal, the yields were 14.9 and 17.9 t/ha, respectively (Table 5). The per cent yield increase in Arka Anoop over check varieties Arka Komal and Contender was 15.85 and 69.00 in that order (Table 5). The yields in the check varieties were comparatively better during *kharif* due to low or no incidence of rust.

The study confirmed that the resistance to rust in french bean variety, Arka Bold was controlled by single dominant gene. Further, it also revealed that the selected breeding line, Arka Anoop was resistant to rust with average yield potential of 19.8 t/ha.

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Radiosensitivity of amla (*Emblica officinalis* Gaertn.) varieties treated with gamma rays

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ABSTRACT

Investigations were carried out at the Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, during 2003-2005 to work out radiosensitivity of five varieties of amla (*Emblica officinalis* Gaertn.) exposed to different doses of gamma rays. Scions of five amla varieties, *viz.*, BSR-1, Kanchan, Krishna, NA-7 and Chakaiya, were irradiated with different doses (1.0 to 5.0 kR) and these were grafted on to rootstocks. Based on the sensitivity study, LD_{50} for 100% survival ranged from 1.0 to 2.0 kR for all the five varieties. All the amla varieties could survive upto 10-20% at lower doses (upto 2.5 kR).

Key words : Amla, sensitivity, LD_{50}

INTRODUCTION

Vegetatively propagated crops are a group of plants highly suitable for application of mutation breeding for various reasons. Continuous vegetative propagation has led to less variability in the amla plant populations. Induction of mutation is considered an important breeding tool to create new variation for economic traits. Moe and Han (1973) stated that improvement of a crop cultivar was usually accomplished by adding one or more desirable attributes to the initial, commercially grown strain and, hence, mutagenesis was the simplest means to achieve desirable breeding objectives.

Induction of mutations in vegetatively propagated plants has been investigated extensively by various authors, from Broertjes to Spiegel Roy. Induction of mutations in amla (*Emblica officinalis* Gaertn.) has been receiving increasing attention recently for crop improvement (Pathak, 2003). Adequate information on sensitivity of different varieties of amla to different doses of gamma rays is not available. The present investigation purports to assess sensitivity of amla to different gamma ray treatments in terms of survival percentage and degree of crop growth inhibition. The degree of growth inhibition in a woody plant like amla was determined by growth characteristics such as height, spread, number of buds or leaves and fresh and dry weight. The traits, *viz.*, fifty per cent bud survival and inhibition of growth, were used as biological parameters to determine sensitivity of amla varieties to different doses of gamma rays.

MATERIAL AND METHODS

The present investigation of induced mutation breeding in amla (*Emblica officinalis* Gaertn.) was undertaken in the Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, from 2003-2005. Improved amla varieties, BSR –1, Krishna, Kanchan, Chakaiya and NA-7 (maintained at the Central orchard of the Horticultural College and Research Institute, TNAU), which can be readily propagated by cleft-grafting, were chosen for the study. A physical mutagen (Gamma rays) was employed in the present study. Amla scions with dormant buds were treated with gamma rays. Scions of pencil-thickness, consisting of 10 nodes (dormant buds) from seven year old mother trees, were collected and treated under a temperature range of 25± 2°C. The scions were stored by wrapping in a wet gunny-cloth at room temperature until treatment, and thereafter, till grafting on to rootstock. The treated scions were cleft-grafted the same day on one-year old amla seedling rootstocks. Both the gamma ray exposed and untreated grafts were planted in pots and these received uniform standard operations after-care.

Sensitivity studies

A preliminary study was conducted to fix the optimal dose of gamma ray irradiation on survival of grafts

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(scions treated with gamma ray). The range of gamma ray (kR) doses as 1.00, 2.00, 3.00, 4.00 and 5.00. Different criteria adopted for assessing sensitivity were:

Graft survival: The survival of the gamma-ray treated grafts was recorded at 30, 60, 90 and 120 days from grafting and expressed as percentage.

Degree of growth inhibition: The degree of growth inhibition was expressed in terms of the following parameters, measured 90 days after grafting:

1. Length of the primary shoot (cm)
2. Number of leaves (90 days from grafting)
3. Fresh weight of the primary shoot (g)

RESULTS AND DISCUSSION

The biological effect of gamma rays (sensitivity) on amla (*Emblica officinalis* Gaertn.) growth and development was studied based on four V_1M_1 growth criteria. The plant parameters studied were: survival, primary shoot length, number of leaves and fresh weight of the primary shoot.

Per cent survival

Percentage of survival of the scions after irradiation showed highly significant differences among different doses of gamma rays. There was progressive reduction in per cent graft-survival, with increase in dose (Table 1). The highest dose of 5.0 kR recorded 16% survival as compared to 92% in the control. The LD_{50} values for survival in the variety BSR-1 ranged from 1.00 to 2.00 kR. However, survival percentage as comparatively low in 'Kanchan' as compared to 'BSR-1' on wild amla rootstock. LD_{50} for survival was reckoned to between 1.00 and 2.00 kR. As registered in the other two varieties, a relatively low survivability of treated scions was observed in different doses of gamma rays of the variety Krishna. Percentage survival ranged from 8.69 to 58.33. Survival percentage of NA-7 amla variety was

Table 1. Survival percentage of amla varieties in V_1M_1 generation following gamma ray irradiation

Variety	Dose of gamma ray (kR)	Survival percentage (%)	Primary shoot length (cm)	Number of leaves	Fresh weight of primary shoot (g)
BSR-1	Control	92.00	21.88	22.00	5.66
	1.0	60(65.22)	26.50(121.12)	26 (118.18)	6.87(121.38)
	2.0	44(47.83)	23.23(106.17)	21(95.45)	6.76(119.40)
	3.0	36(39.13)	16.63(76.01)	19(86.36)	5.55(98.40)
	4.0	24(26.09)	12.50(57.13)	17(77.27)	4.33(76.50)
	5.0	16(17.39)	7.93(36.24)	11(50.00)	2.89(51.06)
Kanchan	Control	96.00	20.26	18.00	5.80
	1.0	56(58.33)	23.50(115.99)	20(111.11)	6.53(112.59)
	2.0	40(41.67)	22.00(108.59)	19(105.56)	6.60(113.80)
	3.0	36(37.50)	14.00(69.10)	16(88.89)	5.45(93.97)
	4.0	24(25.00)	12.00(59.23)	14(77.78)	3.89(67.07)
	5.0	12(12.50)	6.30(31.10)	11(61.11)	2.50(43.10)
Krishna	Control	92.00	21.80	18.00	7.07
	1.0	56(58.33)	21.43(98.30)	18(100.00)	6.00(84.87)
	2.0	40(43.48)	20.90(95.87)	17(94.44)	5.55(78.50)
	3.0	28(30.43)	19.47(89.31)	16(88.89)	4.80(67.89)
	4.0	16(17.39)	16.63(76.28)	12(66.67)	4.30(60.82)
	5.0	8(8.69)	6.53(29.95)	10(55.56)	3.20(45.26)
NA-7	Control	96.00	22.40	20.00	6.25
	1.0	52(54.17)	20.63(92.10)	22(110.00)	7.01(112.16)
	2.0	36(37.50)	19.50(87.05)	18(90.00)	6.45(103.20)
	3.0	28(29.17)	9.65(43.08)	15(75.00)	5.18(82.88)
	4.0	28(29.17)	5.63(25.13)	13(65.00)	5.55(88.80)
	5.0	12(12.50)	3.40(15.18)	11(55.00)	2.87(45.92)
Chakaiya	Control	88.00	23.00	22.00	6.00
	1.0	52(59.09)	22.45(97.61)	24(109.09)	6.85(114.17)
	2.0	36(40.90)	19.53(84.91)	20(90.09)	6.01(100.17)
	3.0	12(13.64)	18.43(80.13)	17(77.27)	6.00(100.00)
	4.0	8(9.09)	12.15(52.83)	13(59.09)	5.30(88.30)
	5.0	8(9.09)	9.68(42.09)	12(54.55)	3.25(53.80)

* Values in parantheses are per cent values over control

found to be inversely related to increasing doses of gamma rays. The LD_{50} sensitivity dose for survival for NA-7 variety ranged from 1.00 to 2.00 kR The highest dose of gamma rays (5.00 kR) recorded 8% survival rate in 'Chakaiya' as compared to 88.88 % in the untreated control. The LD_{50} dose for survival was 1.00 to 2.00 kR.

In general, mutagenic treatments of scions from different amla varieties in the present study resulted in lower percentage of survival. Success of the irradiated scions when grafted depends upon union of cambium layers of the stock and scion and consequent production of normal conducting tissue. Snow (1933) demonstrated that meristematic activity of cambium in the region of graft-union is stimulated by indoleacetic acid, and this view is shared by several researchers. That the level of auxin concentration in plants drops after exposure to ionizing radiation is also well-recognized. Irradiation immediately lowers free-acid auxin levels in the crop plant and the inactivation of auxin generally increases with increasing exposures (Skoog, 1935). In this regard, Gordon and Weber (1955) clearly showed that *in vivo* auxin synthesis was non- exponential with increment in gamma exposure but, that, the extent of inhibition of synthesis increases with increased dose. Moreover, mutagenic treatments cause chromosomal aberrations, which adversely affect cell-division. The lower percentaged of survival of grafts observed after treatment of the scion-wood with gamma rays may be attributed to a drop in auxin levels and to chromosomal aberrations caused by mutagenic treatments.

Further, it was also observed, in the present study, that the survival percentage of amla grafts decreased gradually as the dose of gamma rays increased, but, the decrease was rather sharp at 4 and 5 kR for all the five amla varieties. This was further exemplified by the sensitivity of LD_{50} doses required to cause 50% lethality. According to Viswanathan *et al* (1992), reduced survival per cent at higher doses of gamma radiation may be mainly due to cell death and higher rate of ionization in the nuclei. The drastic decrease in survival percentage under different doses of irradiation may be due to physiological imbalance and damages caused at the molecular level, which results in chromosomal aberrations causing considerable cytological changes.

Primary shoot length

The primary-shoot length on 90^{th} day from grafting of the irradiated scion of BSR-1 variety was lower than that in the control, particularly, at higher doses (3.00, 4.00 and 5.00 kR), but at lower doses (1.00 and 2.00 kR), there was a slight increase in the primary shoot length as compared to the untreated control. LD_{50} values for this trait ranged from 4.00 to 5.00 kR. In the variety 'Kanchan', an increase of 15.99 and 8.59% over the control was recorded in 1.00 and 2.00 kR treatments, respectively whereas, the primary-shoot length at different doses showed a decreasing trend with 3.00, 4.00 and 5.00 kR of gamma rays. The LD_{50} dose of gamma rays for this trait was noticed to be between 4.00 and 5.00 kR. Reduction in primary-shoot length of the treated plants of 'Krishna' showed linearity with the increasing dose of gamma rays. Reduction in the primary-shoot length ranged from 98.3 to 29.95% of control, indicating a drastic reduction for this character at higher doses. Fifty per cent reduction in lethality was obtained between doses of 4.00 and 5.00 kR. Gamma ray irradiated NA-7 amla grafts registered a reduction in primary-shoot length showing an inverse relationship to increase in dose of gamma rays and the reduction ranged from 20.63 to 3.4 cm as dosage increased from 1.00 kR to 5.00 kR. The LD_{50} value for this trait ranged between 3.00 and 4.00 kR. The percentage of reduction ranged from 97.61 to 42.09 in the variety Chakaya. The LD_{50} sensitivity value was observed in the dose range of 4.00 and 5.00 kR.

It is seen clearly that length of the primary-shoot gets gradually reduced in proportion to increase in dose of gamma rays. This reduction in shoot length of amla is considered to be a combined effect of mortality of a few cell initials, delay in sprouting and slow growth-rate. Reduction in growth of mutagen-treated meristems of the shoot is a cumulative expression of at least three different types of cytologically identifiable effects (Evans, 1965).

Positive explanations for the reduction in plant height have been offered for reduced crop growth at different stages following mutagenic treatments, such as auxin destruction (Skoog, 1935), inhibition of auxin synthesis (Gordon, 1954), failure of the assimilatory mechanism (Quastler and Baer, 1950), production of diffusible growth-retarding substances (Mackey, 1951), changes in specific activity of enzymes (Haskins and Chapman, 1956) and inhibition of DNA synthesis (Mikaelson, 1968).

Growth indices of the physiological effects, viz. number of leaves and fresh weight of shoot were also studied.

Number of leaves and fresh weight of primary shoot

Gamma ray treatments in the present study recorded marked inhibitory effect in respect of number of leaves. Fifty per cent reduction in the number of leaves per plant was observed between doses of 4.00 to 5.00 kR in all

the varieties. Inhibition occurred at 3.00 kR for BSR-1, Kanchan and Krishna, and, 3.00 and 5.00 kR for NA-7 and Chakaiya. This may be due to direct effect of the mutagens on the growing points of amla varieties. Depending upon the physiological and developmental stage these might have been killed or inactivated by various doses of the toxic mutagen and, hence, the reduction in number of leaves. However, stimulatory effects of ionizing radiation were obtained for fresh weight of shoot and production of leaf at lower doses of gamma rays. The increase was significant in some cases, but was less in magnitude indicating that such physiological stimulation are is not likely to be exploited on a commercial scale for crop improvement. The basis for stimulatory responses obtained, though small in magnitude, is of significant interest.

In general, there was a clear and perceptible variation in susceptibility of amla varieties to injury by gamma ray. There is a considerable variation in the LD_{50} values and the differences exhibited were greater between levels of the mutagen and between varieties. In most of the cases, no exposure produced those exact levels of survival and hence LD_{50} values were determined by interpolation from the survival-curve. A possible explanation for this differential sensitivity could be that frequency of cells involved in the different treatments may be higher.

Thus, the present study clearly indicated that survival percentage is a reliable criterion for arriving at the optimum dose of irradiation in amla (*Emblica officinalis* Gaertn.). Better survival percentage of plants seen at lower doses may be due to radiation resistant-nature of the biological material upto a certain dose. This is evident from the result that higher doses of the mutagen resulted in poor survival percentage.

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Effect of spacing and corm size on growth, flowering and corm production in gladiolus cv. White Prosperity under Kashmir conditions

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Abstract

A study was carried out during 2005 - 2006 at the Division of Floriculture, Medicinal and Aromatic Plants, SKUAST-K, Shalimar, to determine the effect of corm size (4.1-4.5, 4.6-5.0 and 5.1-5.5 cm) and spacing (10 x 20, 15 x 20 and 20 x 20 cm) on growth, flowering and corm production in gladiolus cv. White Prosperity. Larger-sized corms (5.1-5.5 cm) with wider plant spacing (20 x 20 cm) gave the best performance. Number of days taken to spike emergence, plant height, number of leaves plant^{-1}, spike length, number of florets spike^{-1} and diameter of floret were observed to be significantly better with larger-sized corms. Minimum days taken to slipping were also found to be due to larger size of the corms. Number of corms plant^{-1}, corm weight, diameter of corm, number of cormel plant^{-1} and cormels weight plant^{-1}, in terms of both quality and quantity, showed increasing trend with an increasing corm-size and spacing. Therefore, wider spacing and larger corm size may be recommended for realising better quality and higher production in gladiolus cv. White Prosperity under Kashmir conditions.

Key words: Gladiolus, corm size, spacing, vegetative growth, flower quality

INTRODUCTION

Gladiolus is considered as an easy to grow bulbous ornamental because of its wide adaptability to varying agro-climatic regions. It is grown extensively in the tropical, sub-tropical and temperate regions of the world. Yield as well as quality of flower spikes and daughter corms depends on several factors, of which size of the mother corm and spacing, play an important role. Therefore, the present study was undertaken to work out the optimum size for the mother corm in gladiolus cv. White Prosperity and ideal spacing for the sowing corms under Kashmir conditions.

MATERIAL AND METHODS

Experiments were conducted for two consecutive years (2005 and 2006). Nine treatments were imposed with three corm sizes (dia in cm), viz., 4.1-4.5, 4.6-5.0 and 5.1-5.5 and three plant spacings (cm), viz., 10 x 20, 15 x 20 and 20 x 20 between plants and rows. Corms were planted at a depth of 5 cm in the first week of March during both years. Experiments were laid out in randomised block design with three replications. Observations were recorded on vegetative growth, floral and corm production parameters. Spikes were harvested when the lowermost florets developed colour. Corms were lifted from the soil two months after harvest of spikes. Two years data collected from 5 plants/plot each year were analysed statistically (Chandel, 1975).

RESULTS AND DISCUSSION

Vegetative characters

The results clearly indicate a significant influence of corm size on growth, flowering in gladiolus (Table 1). Bigger corms took significantly less number of days (20.16 and 18.77) to corm emergence, but, per cent corm emergence did not show any significant effect during 2005 and 2006. Bigger sized corms also produced taller plants (71.22 and 73.45 cm) and more number of leaves (7.78 and 8.71) plant^{-1}, as also observed by Mukhopadhyay and Yadav (1984) and Islam *et al.* (2000). This could be due to higher amounts of stored food reserves in large corms.

Out of the three spacings, viz., 10 x 20, 15 x 20 and 20 x 20 cm, the spacing of 20 x 20 cm showed early

Table 1. Effect of corm size and spacing on growth and flowering in gladiolus cv. White Prosperity

Treatment	Days taken to sprouting		% corm sprouting		Plant height (cm)		No. of leaves plant^{-1}		No. of days taken to spike emergence		Spike length (cm)		No. of florets spike^{-1}		Floret diameter (cm)	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
Corm size (cm)																
4.1-4.5	21.38	19.87	98.14	99.10	68.94	71.28	7.06	8.15	81.75	83.33	88.33	90.83	16.50	17.82	9.71	10.52
4.6-5.0	20.66	19.18	99.32	98.15	70.77	73.05	7.43	8.47	80.83	82.33	95.27	98.30	17.00	18.37	10.55	11.30
5.1-5.6	20.16	18.77	98.45	99.25	71.22	73.45	7.78	8.71	79.17	80.83	99.11	101.66	17.83	19.36	10.72	11.62
CD *(P=0.05)*	0.69	0.72	NS	NS	2.01	1.87	0.18	0.18	0.15	0.20	1.96	2.03	0.78	0.79	0.58	0.60
Spacing (cm)																
10 x 20	21.38	19.97	98.17	98.25	69.01	71.75	7.28	8.24	81.15	82.72	87.22	89.72	16.66	18.03	9.55	10.49
15 x 20	20.44	19.00	98.09	97.14	71.05	72.65	7.47	8.30	80.64	82.22	97.27	99.87	16.88	18.33	10.48	11.27
20 x 20	20.38	18.86	99.00	99.12	71.37	73.38	7.51	8.79	79.96	81.55	98.72	101.20	17.77	19.19	10.95	11.78
CD *(P=0.05)*	0.69	0.72	NS	NS	2.01	1.87	0.18	0.18	0.15	0.20	1.96	2.03	0.78	0.79	0.58	0.60

emergence of corms as compared to closer spacings (10 x 20 cm) also corroborated by Langhlans and Smith, 1966. However, the per cent corm emergence was found to the non-significant in different spacings. Number of leaves plant^{-1} (7.51 and 8.79) and plant height (71.37 and 73.31) significantly increased with wider spacing i.e. 20 x 20 cm (Table 1). Maximum plant height resulted from corms planted at a spacing of 20 x 20 cm during both the years. Wider spacing gives more space to the plant to derive nutrients from the soil and reduces competition between plants for nutrients and light (Sujatha and Singh, 1991; Yadav and Singh, 1996). Reduction in plant height under higher densities may be due to greater competition between plants for various factors.

Floral characters

Flower quality was also significantly influenced by corm size. Larger corms produced significantly longer spikes (99.11 and 101.66cm) and maximum number of florets (17.83 and 19.36) spike^{-1} during the years, viz., 2005 and 2006, respectively (Table 1). Spike emergence, number of florets spike^{-1} and diameter of the floret were also reported to increase with increase in size of mother corms, by Mukhopadhyay and Yadav (1984), Yadav and Singh (1996), and, Islam *et al* (2000).

The widest spacing (20 x 20 cm) resulted in maximum spike length (98.72 and101.20 cm), floret diameter (10.95 and 11.78 cm) and number of florets (17.77 and 19.19) spike^{-1} (Table 1). Similar findings have also reported by other workers earlier (Banker and Mukhopadhyay, 1980; Sujatha and Singh, 1991).

Corm and cormel production

Corm and cormel production was significantly affected by different corm grades used in planting. Significantly higher number of corms (2.28 and 2.62) and cormels (36.11 and 43.38) plant^{-1} were produced in a corm size of 5.1-5.5 cm (Table 2). Similarly, weight and size of the corm significantly increased with increase in size of corm at planting. This may also be due to availability of more food material stored in bigger sized mother corms that helped in better plant growth, corm and cormel production. These results are in agreement with earlier

Table 2. Effect of corm size and spacing on corm and cormel production in gladiolus cv. White Prosperity

Treatment	No. of corms plant^{-1}		No. of cormels plant^{-1}		Weight of 10 corms (g)		Weight of cormels plant^{-1} (g)		Diameter of corm (cm)	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
Corm size (cm)										
4.1-4.5	1.91	2.08	28.42	34.88	402.86	400.07	24.73	30.00	5.30	5.70
4.6-5.0	2.08	2.32	31.71	36.77	442.25	447.32	27.70	33.83	5.43	5.83
5.1-5.6	2.28	2.62	36.11	43.38	464.43	480.55	31.05	35.55	5.50	5.90
CD *(P=0.05)*	0.19	NS	2.45	1.43	20.65	29.10	2.05	1.04	0.07	0.13
Spacing (cm)										
10 x 20	1.85	2.26	27.21	30.94	412.66	420.50	24.18	31.11	5.22	5.62
15 x 20	2.03	2.33	32.04	40.38	420.81	442.12	28.22	32.88	5.47	5.87
20 x 20	2.38	2.43	36.98	43.72	476.07	465.42	31.07	34.38	5.53	5.93
CD *(P=0.05)*	0.19	NS	2.45	1.43	20.65	29.10	2.05	1.04	0.07	0.13

findings of Mukhopadhyay and Yadav (1984), Patil *et al* (1995) and Islam *et al* (2000). Widest plant spacing (20 x 20 cm) significantly increased the number of corms (2.38 and 2.43) and cormels (36.98 and 43.72) plant^{-1}, and weight of cormels (31.07 and 34.38 g) plant^{-1} and size of corm (5.53 and 5.93 cm) plant^{-1} during both years of experimentation. Present findings are, thus, in agreement with many earlier workers (Mukhopadhyay and Yadav, 1984; Arora and Khanna, 1987, and, Sujatha and Singh, 1991). The availability of more light for synthesis of photosynthates and more area for better root growth and nutrient absorption in widest spacing may have enhanced the production of bigger corms and cormels. The positive response of wider spacing on corm and cormel production has also been reported by Mukhopadhyay and Yadav (1984) and Patil *et al* (1995).

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DRIS norms for identifying yield-limiting nutrients in sapota (*Manilkara achras* (Mill). Fosberg) cv. Cricketball

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ABSTRACT

Diagnosis and Recommendation Integrated System (DRIS) identified forty-five nutrient expressions as diagnostic norms from data colleted by surveying seventy-four sapota gardens in Karnataka and dividing the whole population into two sub-groups, namely, low and high yielding, during the year 2005-06. These expressions have shown higher variance and lower coefficient of variation found to have greater diagnostic precision, viz., N/K (0.989), Mg/N (0.264), N/Zn (0.117), Mg/K (0.258), Zn/K (8.609), S/Mg (0.666), Mg/Zn (0.031) etc. The Nutritional Balance Index indicated an overall imbalance of nutrients based on the sum of indices, irrespective of the sign. The diagnosis of nutrient imbalance through DRIS indices indicated that potassium, followed by nitrogen, was the most yield-limiting nutrient among major nutrients and as were copper and zinc among micronutrients. In addition, five nutrient ranges were derived using mean and standard deviation as low, deficient, optimum, high and excess for each nutrient to serve as a guide for diagnostic purposes. Optimum N in the leaf ranged from 1.60 to 1.85%, P from 0.10 to 0.13%, K from 1.63 to 1.85%, Ca from 0.54 to 0.74%, Mg from 0.42 to 0.47% and S from 0.28 to 0.37%. Among micronutrients, optimum iron concentration in the leaf ranged from 113 to 161 ppm, Mn from 21-31 ppm, Zn from 14 to 17 ppm and Cu from 5 to 7 ppm for 'Criketball' variety of sapota.

Key words: Sapota, index tissue, nutrient norms, DRIS, Nutritional Balance Index

INTRODUCTION

To feed balanced nutrition to its 1000 million populations, India needs 92 million tonnes of fruits. Sapota constitutes 1.8% of the share of the country's total fruit production, with an annual production of 8.3 lakh metric tonnes (Anon., 2004). As sapota is an evergreen tree producing several vegetative and floral flushes during the year, and consequently fruits, requires a substantial amount of nutrients for maximizing yield and quality. Hence, its nutrient requirements need to be carefully monitored through modern nutrient management strategy, i.e., leaf analysis, for high productivity. It was planned to develop leaf nutrient standards for sapota using the diagnosis and recommendation integrated system (DRIS), which provides a means for simultaneous identification of imbalances, deficiencies and excesses in crop nutrients and ranking them in order of importance (Beaufils, 1973) as no established standards are yet available for this purpose. This methodology was used successfully to interpret results of foliar analysis in crops such as grape (Bhargava and Raghupathi, 1995) and rose (Anjaneyulu, 2006).

MATERIAL AND METHODS

Sample collection

For establishment of standard values or norms through DRIS, 370 leaf samples were collected from seventy-four sapota gardens in Karnataka during 2005-06. At each site, a composite sample of recently matured tenth leaf from the apex was collected as index tissue. Leaf samples were decontaminated following standard methods (Bhargava and Chadha, 1993). Excess water was removed by pressing the leaves between folds of a blotting paper. The petioles were dried in an oven at 75°C for 72 h and powdered in a Cyclotec Mill before storing. The samples were analyzed for different nutrients (except nitrogen) by digesting 1g of the material in di-acid mixture (9:4 ratio of nitric and perchloric acids) using standard analytical methods (Jackson, 1973). Nitrogen was estimated by the micro-kjeldhal method, whereas phosphorus, potassium and sulphur by vanado-molybdate, flame-photometer and turbidometric methods, respectively. Calcium, magnesium and the micronutrients Fe, Mn, Cu and Zn were analyzed

using Atomic Absorption Spectrophotometer (Perkin-Elmer-A-Analyst-200). Thus, a data bank was established for the entire population.

DRIS norms computation

Using DRIS, the whole population was subdivided as high- and low-yielding (Beaufils, 1973) by earmarking 14 tonnes/ha as the cut-off yield among gardens, although Letzsch and Sumner (1984) indicated that the actual cut-off value had little effect on developing norms as long as it was not too low. Each parameter was expressed in as many forms as possible, e.g., N/P, P/N, N´P, etc. and mean values for each nutrient-expression, together with their associated CVs and variances, were then calculated for the two populations. The mean values (in the high-yielding populations) of nutrient-expression were chosen as diagnostic norms. In making the selection, three basic principles were borne in mind: (i) to ensure that norms were based on Gaussian distribution of yield versus nutrient-expression values, otherwise calculated means (norms) for nutrient expressions that might differ from the true values at maximum crop yield. (ii) to select nutrient expressions for which variance ratios were relatively large, thereby, maximizing the potential of such expressions to differentiate between healthy and unhealthy plants. (iii) to select equal number of nutrient expressions for all the nutrients since this was an absolute requirement of the mathematical model (Walworth and Sumner, 1987).

DRIS indices

DRIS provides a means of ordering nutrient ratios into meaningful expressions in the form of indices. DRIS indices were calculated as described by Walworth and Sumner (1987) using the following formula, with example of one nutrient as shown below:

$$N = 1/10[-f\ (P/N)-f(K/N)+f(N/Ca)+f(N/Mg)-f(S/N)-f(Fe/N)+f(N/Mn)+f(N/Zn)-f(Cu/N)+f(N/dw)]$$

$$\text{Where, } f(N/P) = \frac{N/P}{n/p} - 1 \ \Big| \frac{1000}{CV} \quad \text{when } N/P > n/p$$

$$\text{and } f(N/P) = 1 - \frac{n/p}{N/P} \ \Big| \frac{1000}{CV} \quad \text{when } N/P < n/p$$

where N/P : the actual value of the ratio of N and P in the plant under diagnosis

n/p : value of the norm (which is mean value of the high-yielding unit)

CV : coefficient of variation of high yielding population

Similarly, indices for other nutrients have been calculated using appropriate formulae. The absolute sum (positive and negative) values of nutrient indices generate an additional index called the NBI, nutritional balance index (Walworth and Sumner, 1987).

Leaf nutrient guides/standards

By using mean and standard deviation, five petiole nutrient guides/ranges have been derived, viz., deficient, low, optimum, high and excess, for each nutrient. The optimum nutrient range is the value derived from "mean - 4/3SD (standard deviation) to mean + 4/3SD". The range "low" was obtained by calculating "mean - 4/3 SD to mean - 8/3SD" and the value below "mean - 8/3 SD" was considered as deficient. The value from "mean + 4/3 SD to mean + 8/3 SD" was taken as high and the value above "mean + 8/3 SD" was taken as excessive (Bhargava and Chadha, 1993).

RESULTS AND DISCUSSION

Leaf nutrients concentration range

The nutrient concentration in leaf varied in different orchards of sapota. Leaf N concentration varied from 1.26 to 1.97%, with a mean of 1.573%, indicating that nitrogen content did not vary much among different gardens. However, N was low in some low-yielding gardens when compared to the optimum value. Variation in leaf potassium concentration was high compared to nitrogen indicating, that, the former may be low in most of the low-yielding gardens. Similarly, among secondary nutrients, calcium and sulphur showed higher variation in their concentration (Table 1). Similar trend was noticed for micronutrients in the entire population.

DRIS ratio norms

DRIS identified forty-five nutrient expressions as diagnostic norms that have a higher variance and low

Table 1. Mean and range of nutrient concentrations in sapota

Nutrient	Range	Mean
N (%)	1.26 – 1.97	1.573
P (%)	0.05 – 0.18	0.099
K (%)	1.00 – 2.05	1.562
Ca (%)	0.21 –0.94	0.566
Mg (%)	0.32 – 0.53	0.421
S (%)	0.14 – 0.42	0.283
Fe (ppm)	59 – 198	119
Mn (ppm)	10 – 47	21
Zn (ppm)	10 – 27	14
Cu (ppm)	2 – 10	05

Table 2. DRIS ratio norms for sapota

Selected ratios	Norms	C.V.%	Selected ratios	Norms	C.V%
P/N	0.063	32	K/Cu	0.365	28
N/K	0.989	14	Ca/Mg	1.292	25
Ca/N	0.340	29	Ca/S	2.004	30
Mg/N	0.264	16	Fe/Ca	228.6	46
S/N	0.178	29	Ca/Mn	0.028	38
Fe/N	71.71	33	Ca/Zn	0.039	28
Mn/N	13.69	41	Ca/Cu	0.121	40
N/Zn	0.117	14	S/Mg	0.666	19
N/Cu	0.354	25	Fe/Mg	274.7	35
P/K	0.062	32	Mg/Mn	0.022	32
Ca/P	6.067	51	Mg/Zn	0.031	16
Mg/P	4.543	31	Mg/Cu	0.093	28
S/P	3.043	40	Fe/S	434.5	46
Fe/P	1219	39	S/Mn	0.014	35
P/Mn	0.005	40	Zn/S	53.94	36
Zn/P	152.7	34	S/Cu	0.063	37
P/Cu	0.022	38	Fe/Mn	5.892	45
Ca/K	0.336	32	Fe/Zn	8.386	38
Mg/K	0.258	13	Fe/Cu	25.28	41
S/K	0.173	27	Zn/Mn	0.730	34
Fe/K	70.44	36	Mn/Cu	4.862	48
Mn/K	13.34	41	Zn/Cu	3.102	30
Zn/K	8.609	19	—	—	—

coefficient of variation between high-and low-yielding populations (Table 2). Going by basic principles, N/K (0.989), Mg/N (0.264), N/Zn (0.117), Mg/K (0.258), Zn/K (8.609), S/Mg (0.666), Mg/Zn (0.031), involving macro- and micronutrients which have shown lower CV values compared to others, were selected and these ratios might have a greater physiological rationale. Potassium is known to play a key role in N uptake and translocation, whereas Mg and N are vital constituents of chlorophyll (Raghupathi *et al*, 2004). Hence, maintaining correct ratios of these nutrients is obviously important for the quantum of yield in any crop. Maintaining the ratios of some expressions at optimum when they were with large coefficient of variation was much less critical for performance of the crop. Therefore, nutrients considered as yield-building components, need to be maintained in a state of relative balance for each to be utilized with maximum efficiency for dry matter/yield production (Anjaneyulu, 2006).

DRIS indices and NBI

In Table 3, DRIS indices are presented along with the order in which nutrients limited yield. Thus, DRIS simultaneously identified imbalances, deficiencies and excesses in crop nutrients and ranked them in order of importance. DRIS index is a mean of the deviations of ratios containing a given nutrient, from their respective normal or optimum values. As the value of each ratio function was added to one index sub-total and subtracted from another prior to averaging, all indices were balanced around zero. Thus, the nutrient indices that sum up to zero indicate an optimum level, negative values as relative deficiency and positive values as relative excess of that particular nutrient (Mourao Filho, 2004). The absolute sum values of the nutrient indices generated an additional index called the Nutritional Balance Index (NBI) which indicated an overall imbalance of nutrients in each low-yielding orchard, based on the sum of indices, irrespective of sign. Higher the NBI, larger is the plant nutritional imbalance and thus, lower the yield. The yield-limiting nutrients differed from garden to garden, though some of the nutrients were more prominent. Thus, diagnosis of nutrient imbalance through DRIS indices indicated the most yield-limiting nutrient was potassium followed by nitrogen among major nutrients, and, copper and zinc, among micronutrients. Copper was usually not a yield-limiting factor in many fruit crops such as grape, mango, etc. in these areas. However, copper was observed to be a yield limiting factor in most of the low-yielding sapota gardens (Table 3) after potassium, as these gardens did not receive copper fungicidal sprays for disease management.

Leaf nutrient standards

By using mean and standard deviation, five leaf nutrient guides/ranges have been derived as deficient, low, optimum, high and excess, for each nutrient (Table 4). Optimum leaf N for sapota ranged from 1.60 to 1.85%, whereas, the optimum P range was low, indicating a lower requirement of P compared to N. It was observed that P

Table 3. Diagnosis of nutrient imbalance in low =yielding sapota gardens

Most limiting					Optimum		Excess			NBI
K-269	Cu-149	Zn-120	Mn-84	S-38	P12	Fe86	Mg101	N111	Ca350	(Sum)1320
K-196	Cu-99	Zn-57	Mn-54	N-3	P23	S41	Fe57	Mg82	Ca206	818
K-162	Cu-91	Zn-77	N-27	S9	Mn10	P16	Fe52	Mg68	Ca202	714
K-306	Cu-181	Mn-120	N-83	Zn-77	Mg56	S78	Ca123	Fe223	P287	1534
K-359	Zn-130	N-99	Cu-44	Ca19	S33	P36	Mg48	Fe157	Mn339	1264
K-280	N-89	Mn-76	Zn-12	P40	Mg50	Cu51	S72	Ca73	Fe171	914
K-206	Mn-179	Cu-113	N-5	Ca 46	P46	Zn48	Mg49	S139	Fe175	1006
K-83	Cu-78	S-77	P-17	N1	Zn6	Mg14	Fe36	Ca47	Mn151	510

Table 4. Leaf nutrient standards for sapota cv. Cricketball

Nutrient	Deficiency	Low	Optimum	High	Excess
N (%)	<1.34	1.34 – 1.59	1.60 – 1.85	1.86 – 2.12	>2.12
P (%)	<0.06	0.06 – 0.09	0.10 – 0.13	0.14 – 0.17	>0.17
K (%)	<1.40	1.40 – 1.62	1.63 – 1.85	1.86 – 2.10	>2.10
Ca (%)	<0.32	0.32 – 0.53	0.54 – 0.74	0.75 – 0.97	>0.97
Mg (%)	<0.36	0.36 – 0.41	0.42 – 0.47	0.48 – 0.53	>0.53
S (%)	<0.19	0.19 – 0.27	0.28 – 0.37	0.38 – 0.46	>0.46
Fe (ppm)	<65	65 – 112	113 – 161	162– 210	>210
Mn (ppm)	<11	11 – 20	21 – 31	32 – 42	>42
Zn (ppm)	<10	10 –13	14 – 17	17 – 21	>21
Cu (ppm)	<03	3 - 4	5 – 7	8 – 10	>10

was generally much less a limiting factor in sapota production. Requirement for K is always next only to nitrogen, as this nutrient is involved not only in production but also in improving the quality of sapota. Among the gardens surveyed, calcium and magnesium status of many individual gardens was optimum compared to their optimum ranges. Similarly, sulphur was not a yield-limiting factor in most of the gardens. Among micronutrients, copper and zinc were found to be deficient in most of the low-yielding gardens. The concentration of copper was as low as 2 ppm and zinc 10 ppm in many low-yielding gardens. However, iron and manganese were low only in very few gardens. It can be concluded that yield-limiting nutrients in sapota gardens can be corrected by following efficient fertilizer application based on leaf nutrient norms developed.

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Nitrogen use efficiency in tomato (*Lycopersicon esculentum* L.) and French bean (*Phaseolus vulgaris* L.) as influenced by coating of urea with neem oil and graded levels of nitrogen

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ABSTRACT

In a pot-culture study, 'Arka Shrestha' tomato and 'Arka Komal' French bean were raised on red sandy-loam to compare urea coated with neem oil (2% w/w, NOCU) and prilled urea (PU) applied at 60, 80 and 100% of recommended N dose. To facilitate direct measurement of N use parameters, urea enriched with ^{15}N (1 atom per cent excess) was used as the source of N. Compared to 'no urea' control, the application of N significantly increased dry matter production, fruit/pod yield as well as the parameters of N use. Prilled urea coated with neem oil (NOCU) was superior to PU in both the crops and produced 21% and 9% higher yield compared to the latter. Increasing the dose of N significantly increased dry matter production, yield and all parameters of N use. However, the interaction effects showed that N applied as NOCU at 80% the of recommended dose produced fruit/pod yield *at par* with that obtained at 100% of the recommended dose applied as PU in both crops. Corresponding fertilizer utilization achieved was 14.9% and 59.0% when 80% of N was applied as NOCU compared to 11.5% and 30.1 obtained when 100% of N was applied as PU in tomato and French bean, respectively.

Key words: Neem coated urea, nitrogen use efficiency, tomato, French bean

INTRODUCTION

Application of fertilizer nitrogen to soil is subjected to transformation losses due to presence of urease in the soil. To overcome such losses, coating/ blending urea with neem oil / products is a convenient and effective method. Melicans, or bitters, present in neem (*Azadirachta indica* L.) products, when blended with urea, inhibit nitrification and volatilization culminating in reduced leaching losses in soil (Devakumar and Goswami, 2002; Suri *et al*, 2004). Accumulation of ammoniacal and other mineralized nitrogen, owing to microbial immobilization by lowered rates of nitrification in top soil layers, facilitates its availability to the crop subsequently (Singh *et al,* 1989). Since treating urea with neem oil enhances nitrogen-use efficiency of the applied fertilizer and as the red sandy soils of Bangalore exhibit definite activity of urease enzyme, prilled urea coated with neem oil (2% w/w, NOCU) was compared to prilled urea (PU) at different levels of recommended N levels using 'Arka Shreshtha' tomato and 'Arka Komal' French bean in a pot-culture experiment.

MATERIAL AND METHODS

The experimental soil was red sandy-loam (*Typic Haplustalf*) with pH 5.9, organic carbon at 0.3%, cation exchange capacity of 8.7 cmol (p^+)/kg, urease activity of 2.03 µg NH_4^+/g/hr and alkaline permanganate mineralizable N of 220 kg/ha. In a completely randomized factorial design with 3 replications, the first factor consisted of 2 forms of urea: (i) prilled urea (PU) and (ii) urea coated with 2.0% (w/w) neem oil (NOCU). The second factor involved 3 N levels at 60, 80 and 100% of recommended dose for application to the crops. Pots were filled with 10 kg of 4 mm sieved soil, and, 25 day-old tomato seedlings were planted and seeds of French bean sown to raise 2 seedlings in each pot. Superphosphate and muriate of potash had been incorporated in to the soil earlier. Soon after the seedlings established seeds germinated, urea was broadcast on soil-surface. The fertilizer dose given to the crops was 180:150:120 and 80:100:40 N : P : K kg/ha for tomato and French bean crops, respectively. To facilitate direct measurement of N-use, urea enriched with ^{15}N (1 atom per cent excess) was used. In the case of NOCU, the required

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quantity of ^{15}N -enriched urea was blended by triturating in a quartz pestle and mortar. Fruits/pods were harvested from time to time and dry leaves collected to estimate total dry matter. At the last harvest, shoots were parts into stem, leaf and fruit/pod. The root was washed free of adhering soil. All the plant separates were cleaned with tap water, rinsed with distilled water and dried in an oven at 70° C to estimate dry matter and N content. Abundance of ^{15}N was estimated using ratio mass spectrometer (CE Instruments Flash EA-1112 Series Thermoquest). Values for different plant separates were pooled to obtain uptake of N and other parameters of N-use by the crops. The treatment sum of squares was partitioned into Control *vs*. N application, PU *vs*. NOCU and 60 *vs*. 80 *vs*. 100% of recommended N dosage and their interactions were studied as described by Cochran and Cox (1966).

RESULTS AND DISCUSSION

Effect of N application

Both tomato and French bean responded significantly to N application by way of increased (i) fruit and dry matter production, (ii) N content of the plant and (iii) N uptake by the plant (Tables 1 and 2). The positive response to nitrogen application may be attributed to low levels of available nitrogen in the soil.

Table 1. Effect of N application, coating of urea with neem oil and graded doses of N on fruit yield, dry matter and parameters of N use in tomato cv. Arka Shreshtha

Treatment	Fruit yield (g/pot)	Shoot dry matter (g/pot)	N content (%)	N uptake (g/pot)	Ndff (%)	Fertilizer N uptake (mg/pot)	Fertilizer utilization (%)
Control vs. N application							
Control	295.00	40.20	0.410	0.160	-	-	-
N application	441.60	69.50	0.600	0.420	-	-	-
SEm (±)	10.74	1.76	0.008	0.012	-	-	-
CD *(P=0.05)*	23.41	3.84	0.017	0.025	-	-	-
Prilled urea(PU) vs. neem oil coated urea (NOCU)							
PU	399.90	62.70	0.540	0.340	22.10	66.50	10.00
NOCU	483.30	76.30	0.660	0.510	24.60	116.90	17.10
SEm (±)	5.74	0.94	0.004	0.006	0.23	10.46	0.21
CD *(P=0.05)*	17.69	2.90	0.013	0.019	0.71	4.49	0.63
Levels of nitrogen (percentage of recommended dose)							
60% N dose	384.20	63.90	0.510	0.290	19.90	56.30	11.50
80% N dose	429.20	68.90	0.580	0.410	22.10	77.90	11.90
100% N dose	511.50	75.70	0.700	0.530	28.00	140.90	17.20
SEm (±)	7.03	1.15	0.005	0.008	0.28	1.78	0.25
CD *(P=0.05)*	21.67	3.55	0.016	0.023	0.87	5.50	0.77

Table 2. Effect of N application, coating of urea with neem oil and graded doses of N on pod yield, dry matter production and parameters of N use in French bean var. Arka Komal

Treatment	Pod yield (g/pot)	Dry matter (g/pot)	N content (%)	N Uptake (g/pot)	Ndff (%)	Fertilizer N uptake (mg/pot)	Fertilizer utilization(%)
Control vs. N application							
Control	57.40	26.20	1.170	0.340	-	-	-
N application	72.30	38.10	1.390	0.570	-	-	-
SEm (±)	1.39	0.36	0.011	0.009	-	-	-
CD *(P=0.05)*	3.02	0.79	0.024	0.019	-	-	-
Prilled urea(PU) vs. neem oil coated urea (NOCU)							
PU	69.30	35.30	1.310	0.490	23.90	110.50	39.20
NOCU	75.30	41.00	1.470	0.640	25.70	176.60	60.20
SEm (±)	0.74	0.19	0.006	0.005	0.12	2.10	0.66
CD *(P=0.05)*	2.28	0.60	0.018	0.014	0.37	6.46	2.02
Levels of nitrogen (percentage of recommended dose)							
60% N dose	63.20	40.00	1.270	0.520	20.50	111.10	50.90
80% N dose	71.00	37.00	1.390	0.550	24.40	148.60	51.10
100% N dose	82.60	37.40	1.520	0.620	24.70	170.90	47.00
SEm (±)	0.91	0.24	0.007	0.006	0.15	2.57	0.80
CD *(P=0.05)*	2.80	0.73	0.022	0.018	0.45	NS	2.48

Effect of coating urea with neem oil

Between PU and NOCU, the latter produced significantly higher fruit/pod yield, dry matter production, N content, N uptake, Ndff, fertilizer N uptake as well as fertilizer N utilization (Tables 1 and 2). This may be attributed to delayed dissolution and hydrolysis of urea to ammonia by neem oil present in NOCU leading to continuous and steady supply of nitrogen (Singh and Singh, 1989; Vyas *et al*, 1991; and Upadhyay and Patel, 1992). Nitrification of the ammonia evolved was also inhibited by neem oil leading to longer persistence of applied urea resulting in better supply of nitrogen and its utilization by the crop at later stages (Biddappa and Sarkunanan, 1981). According to Prasad *et al* (1999), neem products act as dual-purpose inhibitors of

Table 3. Interaction effect of type of urea and levels of N on fruit/pod weight, dry matter production and parameters of N use in tomato and French bean

Type of urea	Level of N (% recommended dose)					
	Tomato			French bean		
	60	80	100	60	80	100
			Fruit/pod weight (g/pot)			
Prilled urea	343.3	371.70	484.7	62.3	68.60	77.0
Neem oil coated urea (NOCU)	425.0	486.70	538.3	64.1	73.50	88.3
SE m (±)		9.95			1.28	
CD *(P=0.05)*		30.65			3.95	
			Dry matter (g/pot)			
Prilled urea	59.6	59.90	68.3	38.2	34.70	32.9
Neem oil coated urea (NOCU)	67.9	77.90	83.1	41.8	39.30	41.8
SE m (±)		1.63			0.34	
CD *(P=0.05)*		5.02			1.03	
			N content (%)			
Prilled urea	0.48	0.520	0.61	1.23	1.27	1.44
Neem oil coated urea (NOCU)	0.54	0.640	0.79	1.30	1.50	1.60
SE m (±)		0.007			0.010	
CD *(P=0.05)*		0.022			0.031	
			N uptake (g/pot)			
Prilled urea	0.21	0.310	0.41	0.49	0.480	0.52
Neem oil coated urea (NOCU)	0.37	0.500	0.66	0.56	0.630	0.73
SE m (±)		0.011			0.008	
CD *(P=0.05)*		0.033			0.025	
			Ndff (%)			
Prilled urea	19.4	21.60	25.2	19.1	26.00	23.4
Neem oil coated urea (NOCU)	20.5	22.60	30.7	21.9	22.80	25.9
SE m (±)		0.40			0.26	
CD *(P=0.05)*		1.23			0.80	
			Fertilizer N uptake (mg/pot)			
Prilled urea	47.2	58.60	93.7	96.5	125.40	109.5
Neem oil coated urea (NOCU)	65.4	97.30	188.2	125.6	171.70	232.4
SE m (±)		2.52			3.63	
CD *(P=0.05)*		7.78			11.18	
			Nitrogen fertilizer utilization (%)			
Prilled urea	9.6	9.00	11.5	44.3	43.10	30.1
Neem oil coated urea (NOCU)	13.3	14.90	23.0	57.6	59.00	63.9
SE m (±)		0.35			0.34	
CD *(P=0.05)*		1.09			1.03	

both ammonia volatilization and simultaneous nitrification. All these factors facilitated supply of N from NOCU for longer time to the crop, in comparison to PU which dissipated faster in the soil when applied.

Effect of N levels

Among the different levels of N tested in tomato, increasing N dosage significantly improved fruit yield, dry matter production and all parameters of N use, irrespective of the type of urea applied (Table 1). Similar trend was also observed for pod yield and N use parameters in French bean (Table 2).

Interaction effects

In tomato, interaction effects (Table 3) conformed to the main effects. In French bean too, a similar trend was evident. However, it is not clear as to why dry matter production showed a significant decline with increasing levels of N applied as PU. When N was applied as NOCU, dry matter production at 80% level showed a significant reduction of 39.3 g/pot compared to 41.8 g/pot at both 60 and 100% N levels. Neem oil coated urea (NOCU) at 80% level of recommended dose produced fruit/pod yield close to that obtained at 100% of the recommended dose applied as PU in both the crops. Results indicate that coating urea prills with neem oil holds promise reducing fertilizer input considerably, without any loss in yield. This has both economic and ecological implications. Further field studies are suggested to be undertaken before extending the results to growers.

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Effect of shade and integrated nutrient management on biochemical constituents of turmeric (*Curcuma longa* L.)

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ABSTRACT

A field experiment was conducted to study the effect of partial shade, inorganic, organic and biofertilizers on biochemical constituents and quality of turmeric. The study was laid out in split plot design, consisting of two main plots viz., open and shade. The sub-plot treatments consisted of different doses of inorganic fertilizers, organic manures, biofertilizers and growth stimulants constituting of 40 different treatment combinations. The treatment combinations, viz., shade with application of 100 % recommended dose of NPK + 50 % FYM (15 t ha^{-1}) + coir compost (10 t ha^{-1}) + *Azospirillum* (10 kg ha^{-1}) + phosphobacteria (10 kg ha^{-1}) + 3 % panchagavya showed increased total chlorophyll content, total phenol content and registered the highest yield per plot. On the contrary, provision of shade decreased the curing percentage as compared to open condition. Among the quality characters, the highest curcumin (5.57 %) and essential oil (5.68 %) content were registered in the treatment, shade with application of 50 % FYM + coir compost + *Azospirillum* (10 kg ha^{-1}) + phosphobacteria (10 kg ha^{-1}) + 3 % panchagavya.

Key words: Turmeric, shade, chlorophyll, phenol, curcumin, oleoresin, biofertilizers, panchakavya

INRODUCTION

Turmeric (*Curcuma longa* L.) an important spice cum medicinal plant belonging to the family Zingiberaceae is considered to be well acclimatized for growth under low light intensities. A certain degree of shade has a crucial role in affecting the plant growth, yield and quality. Turmeric requires heavy input of fertilizers being a nutrient exhaustive crop (Subramanian *et al*, 2001). In order to present wastage of nutrients, which not only hike cost of production but also pollute environment, it is necessary to adopt a strategy for judicious combination of chemical fertilizers, organic manures and biofertilizers to promote, nurture and facilitate sustainable farming for healthier and economical production. In India, though sufficient research on nutritional aspects of turmeric is available (Venkatesha et al, 1998), studies on the standardization of fertilizer requirement under shaded condition are scanty. With this background, the present investigation was taken up to study the influence of partial shade and integrated nutrient management on the biochemical attributes and yield parameters of turmeric.

MATERIAL AND METHODS

The experiment was conducted at the college orchard, TNAU, Coimbatore during the period 2002-04. The experiment was laid out in split plot design with 40 treatment combinations replicated twice. The genotype CL 147 owing to its superiority for yield and quality under shaded condition was used for the present study. The following are the treatment details,

Main plot

$\mathbf{M_1}$ – Open

$\mathbf{M_2}$ – Shade (Sesban (*Sesbania sesban)* + Castor (*Ricinus communis*))

Sub-plot

$\mathbf{S_1}$ - 100% NPK + 100% FYM (30 t ha^{-1}) (recommended dose – 125: 60: 90 kg NPK ha^{-1})

$\mathbf{S_2}$ - 100% NPK + 50% FYM (15 t ha^{-1}) + coir compost (10 t ha^{-1})

$\mathbf{S_3}$ - 100% NPK + 50% FYM (15 t ha^{-1}) + *Azospirillum* (10 t ha^{-1})

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S_4 - 100% NPK + 50% FYM (15 t ha^{-1}) + phosphobacteria (10 t ha^{-1})

S_5 - 100% NPK + 50% FYM (15 t ha^{-1}) + 3 % panchagavya

S_6 - 100% NPK + 50% FYM (15 t ha^{-1}) + *Azospirillum* (10 kg ha^{-1}) + phosphobacteria (10 kg ha^{-1})

S_7 - 100% NPK + 50% FYM (15 t ha^{-1}) + coir compost (10 t ha^{-1}) + *Azospirillum*

(10 kg ha^{-1}) + phosphobacteria (10 kg ha^{-1})

S_8 - 100% NPK + 50% FYM (15 t ha^{-1}) + coir compost (10 t ha^{-1}) + *Azospirillum*

(10 kg ha^{-1}) + phosphobacteria (10 kg ha^{-1}) + 3 % panchagavya

S_9 - 50% NPK + 50% FYM (15 t ha^{-1}) + coir compost (10 t ha^{-1})

S_{10} - 50% NPK + 50% FYM (15 t ha^{-1}) + *Azospirillum* (10 kg ha^{-1})

S_{11} - 50% NPK + 50% FYM (15 t ha^{-1}) + phosphobacteria (10 kg ha^{-1})

S_{12} - 50% NPK + 50% FYM (15 t ha^{-1}) + 3 % panchagavya

S_{13} - 50% NPK + 50% FYM (15 t ha^{-1}) + *Azospirillum* (10 kg ha^{-1}) + phosphobacteria (10 kg ha^{-1})

S_{14} - 50% NPK + 50% FYM (15 t ha^{-1}) + coir compost (10 t ha^{-1})+ *Azospirillum* (10 kg ha^{-1}) +phosphobacteria (10 kg ha^{-1})

S_{15} - 50% NPK + 50% FYM (15 t ha^{-1}) + coir compost (10 t ha^{-1}) + *Azospirillum* (10 kg ha^{-1}) + phosphobacteria (10 kg ha^{-1})+ 3 % panchagavya

S_{16} - 50% FYM + coir compost (10 t ha^{-1}) + *Azospirillum* (10 kg ha^{-1}) + phosphobacteria (10 kg ha^{-1})

S_{17} - 50% FYM + coir compost (10 t ha^{-1})+ 3 % panchagavya

S_{18} - 50% FYM + coir compost (10 t ha^{-1}) + *Azospirillum* (10 kg ha^{-1}) +phosphobacteria (10 kg ha^{-1}) + 3 % panchagavya

S_{19} - 50% FYM + *Azospirillum* (10 kg ha^{-1}) + phosphobacteria (10kg ha^{-1}) + 3 % panchagavya

S_{20} - Absolute control (without any organic manures & fertilizers)

The experimental plot size was 3 m^2 (2 x 1.5 m) and ridges and furrows were formed at a spacing of 45 x 20 cm. Recommended dose of FYM and digested coir compost (DCC) were applied basally on the ridges and furrows of the respective treatments. Chemical fertilizers were applied in five splits (basal, 30, 60, 90 and 120 days after planting). The seeds of the shade crops *viz*., sesban and castor were sown on the bunds in alternate rows. After 60 days of sowing, the first pruning was done by removing excess shoots and branches to get optimum shade for the growth and development of turmeric. Subsequent pruning was done regularly at an interval of 30days. A shade level of around 25 – 30 per cent was maintained throughout the crop period with the aid of Lux meter. The recommended package of practices was followed uniformly irrespective of the treatments imposed.

Total chlorophyll was estimated by adopting the method of Yoshida *et al* (1971) and expressed as mg g^{-1} of fresh weight. The total phenol content was estimated according to Mallick and Singh (1980) and expressed as mg per g of tissue using to catechol as standard. Soluble protein content was estimated with TCA extract of leaf sample following the method of Lowry *et al* (1957) and expressed in mg g^{-1} fresh weight.

The curing percentage of the rhizome was recorded by using the following formula and expressed in percentage.

$$\text{Curing percentage} = \frac{\text{Weight of the cured rhizome}}{\text{Fresh weight of the rhizome}} \times 100$$

Curcumin content was estimated as per the methods of ASTA (Manjunath *et al*, 1991). The essential oil content was estimated as per the methods described in ASTA (Anon, 1968).

RESULTS AND DISCUSSION

It was observed that all the biochemical parameters expressed an increased trend upto 180 days after planting and decreased thereafter.

i. Total chlorophyll content

The total chlorophyll content varied significantly due to shade and application of fertilizers. The treatment combination M_2S_8 (partial shade + 100 % NPK + 50 % FYM (15 t ha^{-1}) + coir compost (10 t ha^{-1}) + *Azospirillum* (10 kg ha^{-1}) + phosphobacteria (10 kg ha^{-1}) + 3 % panchagavya) showed increased total chlorophyll content 1.589, 1.953 and 1.764 mg g^{-1} in 135, 180 and 225 days after planting respectively. Whereas, it decreased in the treatment M_1S_{20} (open + absolute control) with 1.110, 1.445 and 1.325 mg g^{-1} at all the three stages respectively (Table 1). The increase in chlorophyll content under shaded

condition is an adaptive mechanism commonly exhibited in plants to maintain the photosynthetic efficiency as observed by Attridge (1990). Moreover the inhibition of the chloroplast inhibiting chlorophyllase enzyme may also have lead to greater accumulation of chlorophyll in plants under shaded condition. Hence the increase in biomass production under shade could be substantiated by high level of chlorophyll content (Sreekala, 1999). In early stages of crop growth, increased absorption of nutrient would have caused the assimilation of chlorophyll pigment, which helps in synthesis of photosynthates used for rhizome development (Ramanujam and Jose, 1984). Hence, application of 100% NPK would have caused the accumulation of higher amount of chlorophyll pigment which helped in the synthesis of enhanced amounts of photosynthates which were further utilized for rhizome development.

ii) Total phenol content

Phenols are the physiologically active secondary compounds produced by all higher plants which on deposition in the cell wall regions would directly influence the resistance mechanisms (Bradley *et. al*, 1992). Provision of shade was found to have profound influence on the phenol content in all the stages. Increased score (70.76, 91.03 and 74.13 µg g^{-1}) at 135, 180 and 225 days, respectively was observed in the treatment shade (M_2) compared to open condition . Among the sub plots, the treatment S_8 (100 % NPK + 50 % FYM (15 t ha^{-1}) + coir compost (10 t ha^{-1}) + *Azospirillum* (10 kg ha^{-1}) + phosphobacteria (10 kg ha^{-1}) + 3 % panchagavya) recorded greater value in 135 DAP (105.25 µg g^{-1}), 180 DAP (123.69 µg g^{-1}) and 225 DAP (112.07 µg g^{-1}) (Table 2). Experiments in ginger revealed that incidence of disease were high under open condition compared to shaded / intercropped situation (Jayachandran *et al*, 1991). The probable reason for this may be that the plants grown under shaded condition contain more of essential oil possessing bactericidal and fungicidal properties thereby conferring resistance under shade (Raskin, 1992).

iii) Soluble protein

It increased linearly from third month after planting, reached a peak at sixth month and decreased thereafter. Greater protein content (40.42, 88.88 and 76.93

Table 1. Effect of shade and integrated nutrient management on chlorophyll content (mg g^{-1}) at 135, 180 and 225 days after planting in turmeric

Treatments	Total chlorophyll (mg g^{-1})								
	135 DAP			180 DAP			225 DAP		
	M_1 (Open)	M_2 (Shade)	Mean	M_1 (Open)	M_2 (Shade)	Mean	M_1 (Open)	M_2 (Shade)	Mean
S_1	1.357	1.462	1.410	1.682	1.816	1.749	1.563	1.622	1.593
S_2	1.385	1.489	1.437	1.722	1.850	1.786	1.594	1.648	1.621
S_3	1.328	1.445	1.386	1.673	1.795	1.734	1.551	1.598	1.575
S_4	1.314	1.427	1.371	1.659	1.764	1.712	1.536	1.578	1.557
S_5	1.374	1.475	1.425	1.700	1.823	1.762	1.578	1.632	1.605
S_6	1.460	1.521	1.491	1.761	1.893	1.827	1.614	1.678	1.646
S_7	1.485	1.552	1.519	1.795	1.922	1.859	1.631	1.710	1.671
S_8	1.514	1.589	1.552	1.825	1.953	1.889	1.663	1.764	1.714
S_9	1.290	1.412	1.351	1.642	1.752	1.697	1.522	1.564	1.543
S_{10}	1.187	1.332	1.260	1.552	1.645	1.599	1.411	1.512	1.462
S_{11}	1.350	1.278	1.314	1.485	1.575	1.530	1.362	1.496	1.429
S_{12}	1.258	1.384	1.321	1.617	1.715	1.666	1.491	1.536	1.514
S_{13}	1.421	1.510	1.466	1.745	1.875	1.810	1.608	1.660	1.634
S_{14}	1.474	1.538	1.506	1.782	1.911	1.847	1.622	1.692	1.657
S_{15}	1.508	1.575	1.542	1.811	1.941	1.876	1.648	1.742	1.695
S_{16}	1.238	1.380	1.309	1.608	1.689	1.649	1.477	1.525	1.501
S_{17}	1.159	1.310	1.235	1.523	1.621	1.572	1.375	1.508	1.442
S_{18}	1.274	1.399	1.337	1.622	1.726	1.674	1.509	1.555	1.532
S_{19}	1.224	1.354	1.289	1.582	1.680	1.631	1.453	1.518	1.486
S_{20}	1.110	1.265	1.188	1.445	1.542	1.494	1.325	1.468	1.397
Mean	1.336	1.435	1.385	1.662	1.774	1.718	1.527	1.600	1.563

	135 DAP				180 DAP				225 DAP			
	M	S	M at S	S at M	M	S	M at S	S at M	M	S	M at S	S at M
S Ed	0.007	0.021	0.029	0.029	0.005	0.011	0.016	0.016	0.005	0.015	0.022	0.021
CD (*P=0.01*)	0.421	0.056	0.170	0.079	NS	0.031	0.130	0.043	0.345	0.041	0.142	0.058
CD (*P=0.01*)	0.084	0.042	0.075	0.059	0.061	0.023	0.048	0.032	0.069	0.031	0.058	0.043

NS : Non significant

Table 2. Effect of shade and integrated nutrient management on total phenols (µg g^{-1}) at 135, 180 and 225 days after planting in turmeric

Treatment	Total phenols (µg g^{-1})								
	135 DAP			180 DAP			225 DAP		
	M_1 (Open)	M_2 (Shade)	Mean	M_1 (Open)	M_2 (Shade)	Mean	M_1 (Open)	M_2 (Shade)	Mean
S_1	74.65	77.10	75.88	87.77	91.24	89.51	73.53	78.48	76.01
S_2	81.47	83.64	82.56	93.26	103.64	98.45	86.66	87.74	87.20
S_3	72.24	73.90	73.07	82.25	88.28	85.27	68.74	72.59	70.67
S_4	69.10	70.29	69.70	78.40	85.55	81.98	62.44	69.98	66.21
S_5	79.35	82.25	80.80	91.47	98.47	94.97	79.24	83.33	81.29
S_6	90.20	93.60	91.90	99.14	111.11	105.13	95.47	98.45	96.96
S_7	97.26	100.00	98.63	107.58	121.69	114.64	100.03	106.63	103.33
S_8	103.25	107.25	105.25	117.52	129.85	123.69	107.88	116.25	112.07
S_9	62.25	66.25	64.25	74.42	81.14	77.78	59.88	63.21	61.55
S_{10}	42.25	45.99	44.12	57.14	69.45	63.30	45.28	50.78	48.03
S_{11}	36.00	42.20	39.10	53.21	63.18	58.20	40.23	43.95	42.09
S_{12}	53.35	57.38	55.37	68.52	76.98	72.75	52.75	54.77	53.76
S_{13}	86.25	90.48	88.37	95.83	107.58	101.71	91.22	92.22	91.72
S_{14}	93.45	96.30	94.88	102.24	118.50	110.37	98.54	102.58	100.56
S_{15}	100.00	103.65	101.83	112.33	125.14	118.74	103.69	111.11	107.40
S_{16}	50.00	34.65	42.33	65.99	73.65	69.82	48.52	53.27	50.90
S_{17}	38.29	44.26	41.28	54.44	65.21	59.83	40.85	47.99	44.42
S_{18}	59.25	62.48	60.87	70.10	79.36	74.73	57.14	59.47	58.31
S_{19}	46.65	50.59	48.62	62.24	70.10	66.17	46.25	51.11	48.68
S_{20}	33.90	33.00	33.45	48.57	60.47	54.52	36.55	38.77	37.66
Mean	68.46	70.76	69.61	81.12	91.03	86.08	69.74	74.13	71.94

	135 DAP				180 DAP				225 DAP			
	M	S	M at S	S at M	M	S	M at S	S at M	M	S	M at S	S at M
S Ed	0.378	1.838	2.561	2.599	0.416	1.842	2.573	2.606	0.522	1.602	2.269	2.265
CD (*P=0.01*)	NS	4.984	NS	7.048	26.460	4.997	11.070	7.066	33.230	4.344	13.470	6.144
CD (*P=0.05*)	4.801	3.720	5.779	5.260	5.282	3.729	5.926	5.274	6.634	3.242	5.876	4.585

NS : Non significant

mg g^{-1}) was recorded in the treatment, open + 100 per cent NPK + 50 per cent FYM (15 t ha^{-1}) + coir compost (10 t ha^{-1}) + *Azospirillum* (10 kg ha^{-1}) + phosphobacteria (10 kg ha^{-1}) + 3 % panchagavya (M_1S_8) at 135, 180 and 225 days after planting respectively. While the treatment M_2S_{20} (shade + absolute control) exhibited the lowest values (Table 3). Generally soluble protein content is a measure of Rubisco activity in plants and the lower content of soluble protein in shade can be reflected on the lower activity of Rubisco carboxylase (Broadman, 1977).

Yield per plot

Combined application of shade + 100 % NPK + 50 % FYM (15 t ha^{-1}) + coir compost (10 t ha^{-1}) + *Azospirillum* (10 kg ha^{-1}) + phosphobacteria (10 kg ha^{-1}) + 3 % panchagavya showed the highest per plot yield (19.20kg) which was nearly one and half times the absolute control (Table 4). Turmeric being a nutrition exhaustive crop, a linear increase in fresh rhizome yield was recorded with increased levels of NPK and organic manures. Response to fertilizer application was the highest under shade as compared to open condition. The increased response of nutrients under shade may be due to higher photosynthetic efficiency and better partitioning of assimilates. The increased yield due to increased dose of fertilizers was in agreement with previous works of Balashanmugam and Chezhiyan (1986) in turmeric. Increased values for rhizome characters in shade might be due to increased translocation of nutrients from the source and conversion as carbohydrates to the sink through glycolytic pathway (Bisht *et al*, 2000). Combined application of inorganic and organic amendments resulted in increased number and weight of mother rhizomes. Similar conclusions were derived by Maheswarappa *et. al.*(1997).

Curing percentage

The curing percentage exhibited significant differences under open and shaded condition. The treatment M_1S_{18} (open + 50% FYM + coir compost (10 t ha^{-1}) + *Azospirillum* (10 kg ha^{-1}) + phosphobacteria (10 kg ha^{-1}) + panchakavya (3%) (Soak + Spray)) recorded the highest curing percentage (26.76 %) and the treatment M_2S_{20} (shade

Table 3. Effect of shade and integrated nutrient management on soluble protein (mg g^{-1}) at 135, 180 and 225 days after planting in turmeric

Treatment	Soluble protein (mg g^{-1})								
	135 DAP			180 DAP			225 DAP		
	M_1 (Open)	M_2 (Shade)	Mean	M_1 (Open)	M_2 (Shade)	Mean	M_1 (Open)	M_2 (Shade)	Mean
S_1	30.37	28.43	29.40	79.64	75.28	77.46	69.29	61.72	65.51
S_2	36.19	34.14	35.17	81.27	77.69	79.48	71.64	63.75	67.70
S_3	36.26	34.41	35.34	78.56	74.39	76.48	68.49	59.39	63.94
S_4	35.07	32.35	33.71	77.92	73.47	75.70	67.23	59.10	63.17
S_5	37.51	34.72	36.12	80.74	76.49	78.62	70.84	62.86	66.85
S_6	39.14	36.56	37.85	84.24	78.84	81.54	73.26	65.74	69.50
S_7	39.34	37.10	38.22	86.95	80.74	83.85	75.13	66.95	71.04
S_8	40.42	37.38	38.90	88.88	82.39	85.64	76.93	68.95	72.94
S_9	30.90	28.56	29.73	76.69	73.12	74.91	66.47	58.78	62.63
S_{10}	28.62	25.58	27.10	72.47	67.48	69.98	62.83	55.12	58.98
S_{11}	27.15	24.24	25.70	68.95	64.26	66.61	60.5	52.74	56.62
S_{12}	29.61	26.42	28.02	75.74	71.64	73.69	64.28	57.12	60.70
S_{13}	35.54	32.40	33.97	82.86	78.13	80.50	72.84	64.82	68.83
S_{14}	39.86	37.12	38.49	85.23	79.36	82.30	74.37	66.10	70.24
S_{15}	40.38	37.27	38.83	87.36	81.49	84.43	75.84	67.49	71.67
S_{16}	30.01	27.13	28.57	74.89	70.42	72.66	64.01	56.37	60.19
S_{17}	27.21	25.24	26.23	70.49	65.38	67.94	61.65	54.91	58.28
S_{18}	30.21	27.22	28.72	76.14	72.84	74.49	65.99	57.96	61.98
S_{19}	28.14	25.45	26.80	74.10	69.49	71.80	63.75	55.96	59.86
S_{20}	25.26	23.60	24.43	66.04	61.40	63.72	59.10	50.26	54.68
Mean	33.36	30.77	32.06	78.46	73.72	76.09	68.22	60.30	64.26

	135 DAP				180 DAP				225 DAP			
	M	S	M at S	S at M	M	S	M at S	S at M	M	S	M at S	S at M
S Ed	0.129	1.036	1.434	1.466	0.109	1.003	1.387	1.418	0.122	1.219	1.684	1.723
CD (*P=0.01*)	8.222	2.810	4.599	3.975	NS	2.720	4.281	3.846	7.796	3.305	5.110	4.673
CD (*P=0.05*)	1.641	2.097	3.027	2.966	1.382	2.030	2.898	2.870	1.556	2.466	3.504	3.488

NS : Non significant

+ absolute control) with the least score (15.42 %) (Fig 1). This indicated the influence of environment on curing percentage. On the contrary, fresh rhizome yield was more under partial shade. This may be due to higher amount of moisture present in the rhizomes resulting in plumpy rhizomes with lower curing percentage and thereby lower recovery of cured produce, while higher curing percentage in open may be due to production of slender rhizomes with low moisture content. Moreover the addition of organic manures along with biofertilizer combination would have resulted in increased nutrient uptake resulting in greater dry weight of rhizomes. Similar conclusion was obtained by Latha *et al* (1995) in turmeric.

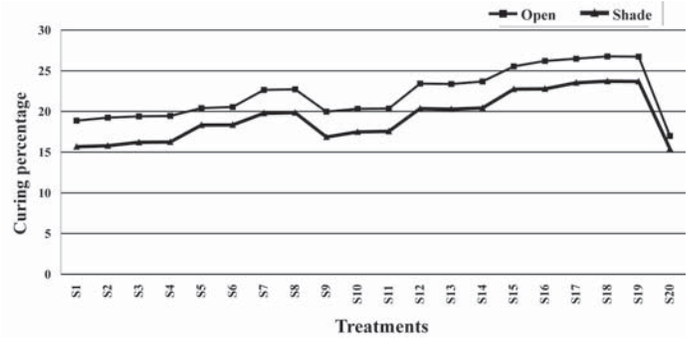

Fig. 1. Effect of shade, inorganic, organic and bio fertilizers on curing percentage in turmeric genotype CL 147

Quality parameters

Curcumin and essential oil

Highest curcumin (5.57 %) and essential oil (5.68 %) content were registered in the treatment M_2S_{18} (shade + 50 % FYM + coir compost + *Azospirillum* (10 kg ha^{-1}) + phosphobacteria (10 kg ha^{-1}) + 3 % panchagavya). The lowest values were documented in the treatment M_1S_{20} (open + absolute control) (Table 4). The increased synthesis and content of curcumin under shade might be due to the increased activity of PAL (Phenyl Ammonia Lyase), the key enzyme involved in curcumin biosynthesis (Chempakam *et al*, 2000). The nitrogen concentration of rhizome expressed a significant positive correlation and K concentration showed negative correlation with curcumin content (Kumar *et al*, 1992). The present findings are in agreement with the earlier work of Upadhayay and Misra (1999) who opined that greater uptake of nutrients increased the essential oil content of turmeric rhizomes.

Table 4. Effect of and integrated nutrient management on rhizome yield per plot (kg), curcumin (per cent) and oleoresin (%) content in turmeric

Treatment	Rhizome yield per plot (kg)			Curcumin (%)			Essential oil (%)		
	M_1 (Open)	M_2 (Shade)	Mean	M_1 (Open)	M_2 (Shade)	Mean	M_1 (Open)	M_2 (Shade)	Mean
S_1	14.31	15.85	15.08	4.23	5.07	4.65	4.41	5.12	4.77
S_2	14.72	16.44	15.58	4.40	5.16	4.78	4.60	5.28	4.94
S_3	14.24	15.30	14.77	4.18	5.00	4.59	4.30	5.04	4.67
S_4	13.70	15.19	14.44	4.16	4.98	4.57	4.13	5.00	4.57
S_5	14.44	15.92	15.18	3.95	4.86	4.41	3.86	4.90	4.38
S_6	14.57	17.14	15.86	4.46	5.20	4.83	4.65	5.34	5.00
S_7	16.03	17.70	16.86	4.42	5.18	4.80	4.62	5.30	4.96
S_8	16.60	19.20	17.90	4.77	5.40	5.09	4.88	5.50	5.19
S_9	13.53	15.06	14.30	4.18	4.98	4.58	4.19	5.02	4.61
S_{10}	12.48	13.27	12.87	4.00	4.88	4.44	3.91	4.91	4.41
S_{11}	11.80	13.20	12.50	4.02	4.88	4.45	3.98	4.93	4.46
S_{12}	13.07	14.01	13.54	3.92	4.85	4.39	3.84	4.87	4.36
S_{13}	14.58	17.09	15.84	4.22	5.04	4.63	4.36	5.08	4.72
S_{14}	15.56	17.47	16.51	4.80	5.42	5.11	4.90	5.53	5.22
S_{15}	16.55	19.09	17.82	4.80	5.50	5.15	4.91	5.57	5.24
S_{16}	12.95	13.97	13.46	4.81	5.51	5.16	4.95	5.62	5.29
S_{17}	12.02	13.14	12.58	4.38	5.14	4.76	4.56	5.25	4.91
S_{18}	13.18	14.63	13.90	4.82	5.57	5.20	5.00	5.68	5.34
S_{19}	12.62	13.81	13.22	4.50	5.24	4.87	4.69	5.38	5.04
S_{20}	11.27	12.28	11.78	3.84	4.75	4.30	3.72	4.80	4.26
Mean	13.91	15.49	14.70	4.34	5.13	4.74	4.42	5.21	4.81

	Rhizome yield per plot				Curcumin				Essential oil			
	M	S	M at S	S at M	M	S	M at S	S at M	M	S	M at S	S at M
S Ed	0.182	0.520	0.740	0.736	0.007	0.007	0.012	0.010	0.007	0.013	0.019	0.018
CD (*P=0.01*)	11.61	1.411	4.749	1.995	0.427	0.019	0.264	0.027	0.422	0.034	NS	0.049
CD (*P=0.05*)	2.319	1.053	1.978	1.489	0.085	0.014	0.065	0.020	0.084	0.026	0.063	0.036

NS : Non-significant

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Response of garlic to organic and inorganic fertilizers

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ABSTRACT

An experiment was carried out to study the response of organic and inorganic fertilizers on growth, yield and quality of garlic (*Allium sativum* L.) cv. Yamuna Safed-3. The results revealed that the combined application of 25% RDF with 75% N through FYM @ 20 t/ha gave higher marketable bulb yield of 19.34t/ha as compared to other treatments which were statistically on par with 100% RDF (18.53 t/ha) and 50% RDF + 50% N supplied as FYM (18.94 t/ha). It is suggested that for better biometric observations, bulb characters and marketable bulb yield in garlic, combined use of inorganic and organic source of nutrient supply is preferable.

Key words: Organic, biometric, garlic

INTRODUCTION

India is the largest producer of garlic in the world with an annual production 5,65,000 tones at an average productivity of 4.74 t /ha (Shanmugasundaram. 2005), which is much lower than the potential productivity. Garlic, being a nutrient loving crop, responds well to added fertilizers in the soil. Warade *et al* (1995) stated that continuous application of inorganic fertilizers deteriorate the soil. Therefore, to maintain soil fertility in order to supply plant nutrients in balanced proportion for optimum growth, yield and quality of crop, under different agro-ecological situations an integrated use of inorganic and organic source of plant nutrients is to be practiced. Keeping this in view, an experiment was conducted to study the response of organic and inorganic fertilizers on growth, yield and quality of garlic.

MATERIAL AND METHODS

A field experiment was carried out at Department of Horticulture, Marathwada Agricultural University, Parbhani. Maharashtra, India during *rabi* 2005 on response of organic and inorganic fertilizers on garlic, variety Yamuna Safed - 3 by adopting Randomized Block Design with eight treatments viz., T_1-100 % Recommended dose of fertilizers (RDF),T_{2-} 50 % RDF + 50 % N through vermicompost, T_3 -25 % RDF+ 75% N through vermicompost, T_4 -50 % RDF + 50 % N through neem cake, T_5 -25 % RDF +75 % N through neem cake, T_6 -50 % RDF +50 % N through FYM, T_7 -25 % RDF +75 % N through FYM and T_8 –Control (no manures and fertilizers). The soil was medium black with pH 7.6 containing 0.74 % Organic Carbon, 255.02 kg / ha N, 18.32 kg/ha P_2O_5, 327.68 kg/ha K_20. The garlic variety Yamuna Safed-3 was (clove) planted at 15 x 7.5 cm spacing in 1.95 m x 1.35 m plots. The organic manures were applied 10 days before sowing. The inorganic chemical fertilizers, as per the above treatments, were applied through urea, single superphosphate and muriate of potash. Growth parameters were recorded 30, 45, 60, 90, 105 and 120 days after planting (DAP). Statistical analyses of biometrical characters were done following Panse and Sukhatme (1967).

RESULTS AND DISCUSSION

The data on the plant height number of leaves, bolting percentage, days to maturity, neck thickness, polar diameter, equatorial diameter, and shape index are presented in Table 1 which revealed that there were significant differences between organic and inorganic fertilizer treatments. At 120 DAP, the maximum plant height of 71.90 cm was observed in the treatment 25 % RDF+75 % N through FYM and found significantly higher than the control, closely followed by application of 100 % RDF (19.64 cm) and 25 % RDF + 75% N through neem cake (71.34 cm). Treatments T_1, T_2, T_3, T_4, T_5, T_6 AND T_7 did not show significant differences. The control (T_8) showed the lowest plant height (70.06 cm) which may be due to addition of no organic or inorganic fertilizers. Similar finding was reported by Waghachaure (2004) in onion.

Table 1. Effect of organic and inorganic fertilizers on biometric characters of garlic cv. Yamuna Safed-3

Sl. No.	Treatment	Plant height (cm) at 120 DAP	No. of leaves at 120 DAP	Bolting (%)	Maturity (days)	Neck thickness (cm)	Polar diameter (cm)	Equatorial diameter (cm)	Shape index
T_1	100 % RDF	71.64	10.26	21.19	127.33	1.24	4.5	5.1	0.89
T_2	50 % RDF+50% N through vermicompost	71.12	10.33	24.04	130.67	1.16	4.0	4.5	0.84
T_3	25 % RDF+75 % N through vermicompost	71.00	10.20	27.34	132.33	1.13	3.8	4.4	0.86
T_4	50 % RDF+ 50 % N through neem cake	71.26	10.20	24.00	130.33	1.10	4.4	4.9	0.88
T_5	2 5% RDF+ 75 % N through neem cake	71.34	10.13	28.99	129.67	1.04	4.0	4.7	0.86
T_6	50 % RDF+ 50 % N through FYM	70.96	10.40	19.89	126.10	0.99	4.5	5.1	0.90
T_7	25 % RDF+ 75 % N through FYM	71.90	10.80	17.70	126.67	0.93	4.7	5.2	0.90
T_8	Control	70.06	9.73	37.68	133.00	1.22	3.6	4.4	0.81
	SE ±	0.50	0.09	0.84	1.52	0.04	0.07	0.07	0.01
	CD*(P=0.05)*	1.58	0.29	2.56	4.60	0.14	0.22	0.24	0.04

Significantly higher number of leaves per plant (10.80) at 120 DAP was produced under the treatment 25% RDF + 75% N through FYM as compared to other treatments. The second best treatment in this regard was T_6. Treatments T_1, T_2, T_3, T_4, T_5 and T_6 were statistically on par with each other. The lowest number of leaves per plant (9.73) was observed in the control. As regards bolting percentage, it was observed that the treatment T_7 (25% RDF+ 75 % N through FYM) showed a value of 17.70%. Significantly higher bolting (37.68%) was observed in the treatment T_3 Treatment T_6 recorded the earliness in bulb maturity (126.10 days) and found to be significantly higher than the treatment T_3 and T_8 control. The next best treatment for attaining early maturity was treatment T_7 (25% RDF + 75 % N – through FYM) (126.67 days), which was statistically at par with the treatments T_1, and T_5.

The treatment T_8 (control) took maximum days (133.00 days) for bulb maturity. The lowest neck thickness (0.93 cm) in garlic bulbs was recorded in the treatment T_7. Maximum neck thickness in garlic bulbs (1.24 cm) was recorded in the treatment T_1, which was found statistically at par with the treatments T_2, T_3 and T_8. As regards polar diameter, maximum polar diameter of bulb (4.7 cm) was recorded in the treatment T_7, which was significantly higher than the other treatments except T_1 and T_6. Significantly minimum polar diameter (3.6cm) was recorded in the treatment control (T_8).

Similar trend was observed in respect of equatorial diameter of garlic bulbs. Maximum equatorial diameter of bulb (5.2 cm) was recorded in the treatment T_7 (25% RDF+75% N through FYM) the lowest equatorial diameter of bulb (4.4 cm) as found in the treatments T_3 (25% RDF+ 75 % N through vermicompost) and were statistically similar with each other.

Highest shape index of garlic bulb (0.90) was recorded in the treatment T_6 and T_7 while significantly lowest shape index of bulb (0.81) was recorded in the treatment control T_8. Thus, positive influence of combined treatment T_7 on biometric characters of garlic could be attributed due to solubilization of plant nutrients exerted by addition of farm yard manure on native and applied plant nutrients as well as chelating effect on metal ions leading to subsequent uptake of NPK by plant (Subbiah *et al*, 1982). Further, FYM might have enhanced the use efficiency of chemical fertilizer.

The data on bulb characters and yield of garlic shown in the Table 2. Significant differences in respect of fresh weight of bulb and cured weight of bulb were observed in treatments receiving organic and inorganic fertilizers. Maximum fresh weight of bulb (35.60 g) was recorded in the treatment T_7, which was significantly higher than other treatments except treatment T_6. The treatments T_2, T_3, T_5 and T_8 were statistically at par with each other. Significantly lower fresh weight of bulb (24.00 g) was recorded in the treatment T_8. The trend observed in respect of cured weight of bulb (33.40 g) was also similar in the treatment T_7, which was significantly higher than other treatments except the treatments T_1 and T_6. Significantly lower cured weight of bulb (20.40 g) was recorded in the treatment T_8. Similar finding was reported by Lal *et al* (2004) in onion. The maximum number of cloves per bulb (22.00) was recorded in the treatment T_8 followed by T_3 (20.00) and T_2 (19.00).

Table 2. Response of organic and inorganic fertilizer on biometric observations of garlic cv. Yamuna Safed-3

Sr. No.	Treatment	Mean fresh bulb wt (g)	Mean cured weight of bulb (g)	Mean number of cloves /bulb	Length of clove(cm)	Diameter of clove (cm)	Weight of clove / bulb (g)	Bulb yield / plot (kg)	Bulb yield / ha(q)
T_1	100 % /RDF	32.73	30.26	14.00	3.00	1.30	2.13	4.95	188.26
T_2	50 % TDF + 50 % N through vermicompost	29.44	27.17	19.00	2.26	1.21	1.40	4.67	177.15
T_3	25 % RDF + 75 % N through vermicompost	28.61	25.46	20.00	2.40	1.09	1.26	4.63	175.50
T_4	50 % RDF + 50 % N through neem cake	31.46	29.06	15.00	2.90	1.27	1.93	4.77	180.81
T_5	25 % RDF + 75 % N through neem cake	30.93	28.66	17.00	2.70	1.24	1.66	4.77	180.68
T_6	50 % RDF + 50 % N through FYM	34.33	31.93	14.00	3.20	1.32	2.40	4.97	189.33
T_7	25 % RDF + 75 % N through FYM	35.60	33.40	12.00	3.50	1.35	2.73	5.10	193.31
T_8	Control	24.00	20.40	22.00	2.30	1.08	0.88	4.50	170.47
	SE ±	0.90	1.09	16.00	0.01	0.30	0.11	0.05	2.00
	CD *(P=0.05)*	2.75	3.30	1.04	0.30	0.10	0.34	0.18	6.07

The lowest number of cloves per bulb (12.00) was found in the treatment T_7. As regards clove length, maximum length of clove (3.50cm) was recorded in the treatment T_7. The treatments T_2 (2.60), T_3 (2.40) and T_5 (2.70) were statistically at par with each other. The minimum length of clove was measured in the control treatment T_8 (2.30 cm). Maximum diameter of clove (1.35 cm) was recorded in the treatment T_7. Significantly lower clove diameter (1.08 cm) was recorded in the control. As regards clove weight, maximum mean weight of clove was recorded in the treatment T_7 (2.73 g), which was significantly superior to the rest of the treatments except treatment T_6. The treatments T_2 (1.40g), T_3 (1.26 g) and T_5 (1.66 g) were statistically at par with each other. Significantly lower weight of clove (0.88g) was recorded in the treatment T_8. Highest bulb yield per plot was recorded in the treatment T_7 (5.10 kg) followed by the treatment T_6 (4.97 kg) and treatment T_1 (4.95 kg), which were significantly superior over to rest of the treatments under study. The lowest bulb yield per plot (4.50 kg) was recorded in the treatment T_8. As regards yield per hectare, the treatment T_7 recorded the highest bulb yield (19.33 q / ha). The treatments T_1 and treatment T_6 were statistically at par with the treatment T_7. Lowest bulb yield (17.05 t/ha) was recorded in the treatment T_8. Similar results were reported by Shamra *et al* (2003) in onion.

Biometric observations as well as bulb characters and yield of garlic were significantly influenced by the combined use of inorganic chemical fertilizers with organic sources of nutrients. This might be due to gradual and steady release of nutrient during the growth period as well as enhanced biological activity and proper nutrition to the crop (Nair and Peter, 1990 ; Sharma and Bhal, 1995; Hangarge *et al*, 2001). Thus, for better biometric and bulb character and marketable yield of garlic, combined use of inorganic and organic sources of nutrient supply is suggested.

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Oxidative stress and changes in antioxidant and biochemical constituents in papaya (*Carica papaya* L.) under salt stress

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ABSTRACT

Six papaya cultivars viz., Pusa Dwarf, Surya, Solo, CO5, Tainan and Red Lady were subjected to saline water salt stress continuously for a period of six months with saline water irrigation having an EC value of 0.6, 2.0 and 4 dsm^{-1}. Among these, Red Lady was more sensitive while Tainan resisted salt stress. Under salt stress of 4 dsm^{-1}, yield reduced by 10% in Tainan and by 24% in Red Lady compared to unstressed controls. T.S.S. measurement showed that quality of fruits was not affected by saline irrigation in both cvs. Malondialdehyde levels estimated after six months period of stress, as thiobarbituric acid reacting substances, did not increase in Tainan in contrast to substantial increase in Red Lady under stress conditions. There was substantial increase in levels of antioxidant compounds namely, carotenoids, phenols and flavonoids in Tainan compared to Red Lady. In Tainan there were significant increases in reducing and total sugars and sucrose under conditions of stress in contrast to sharp decreases in Red Lady. Under conditions of stress, there was considerable accumulation of total and reducing sugars and sucrose, across the varieties, possibly contributing to osmotic adjustment. Association of salt stress tolerance in Tainan with soluble sugar accumulation could be used as a breeding tool for selecting salt tolerant papaya genotypes.

Key words : Oxidative stress, antioxidants, salt stress, *Carica papaya*

INTRODUCTION

Salinity is a major abiotic stress adversely affecting productivity and quality. Papaya (*Carica papaya* L.) is next only to mango as a rich source of pro-vitamin A (Subhas Chander and Rao, 2004). Growth of certain papaya cultivars under salt stress and some biochemical parameters associated with salt stress tolerance were studied and the results are reported.

MATERIAL AND METHODS

Six papaya cultivars, viz., Pusa Dwarf, Surya, Solo, CO5, Tainan and Red Lady were subjected to salt stress continuously for six months with saline water irrigation having EC value of 0.6, 2.0 and 4.0 dsm^{-1} during the year 2004-05. Among the six varieties, cv. Red Lady was more sensitive to salt while cv. Tainan was resistant to salt stress by excluding the sodium cation from the plant system. On this basis, these two cultivars were selected for further biochemical analysis.

Sixth leaf from the top of the tree in these two cultivars (Red lady and Tainan) was taken for biochemical analysis after 20 saline irrigations imposed at intervals of 10 days. The cleaned samples were cut into 0.5 cm squares, mixed thoroughly and dried at 60°C in an oven. Dried samples were powdered in a mixer and stored for biochemical analysis. Estimation was done on a duplicate set of samples. Oxidative stress in the samples due to salt stress was measured as a change in malondialdehyde content, estimated at six months of stress, as thiobarbituric acid reacting substances (TBARS) as described by Egert and Tevini (2002). Five hundred mg of control and stressed samples was extracted with 10 ml of a mixture of 10 ml 5% aqueous TCA and 1 ml of 0.05% methanolic BHT. The homogenate was centrifuged and 2 ml of supernatant was mixed with 4 ml of saturated solution of TBA. The mixture was heated in a boiling water for bath 30 min, cooled and centrifuged and TBARS measured at 532 nm in a spectrophotometer. 0.5 g control and stressed samples of both cultivars were repeatedly extracted with AR grade acetone, filtered and combined and made upto 100ml. The acetone extracts were directly used for estimation of total

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carotenoids as described by Egert and Tevini (2002). Similarly 80% ethanol extracts of the samples were prepared and 50 ml portions of those extracts were defatted by extraction with hexane thrice. Total phenols were estimated in defatted extracts as per the method described by Sadasivam and Manickam (1996). Flavonoids were estimated in the same extracts by the method of Kim *et al* (2003). The undefatted 80% alcohol extracts were used to estimate soluble carbohydrates. Reducing sugars and total sugars after inversion and, sucrose specifically, were estimated as described by Ashwell (1957).

RESULTS AND DISCUSSION

The response of cvs. Tainan and Red Lady to saline water irrigation is shown in Table 1. Average fruit weight was 1.3 kg in 'Red Lady' and 1.4 kg in 'Tainan'. The yield reduced by 10% in cv. Tainan and 24% in cv. Red Lady, when salt stress was 4.0 dsm^{-1}, compared to the unstressed control. However, quality of fruit was not affected by saline irrigation in both the cvs. as evidenced from TSS data. The yield parameters and quality in both cvs. did not differ significantly from control when salt stress was 2 dsm^{-1}. CV values reveal considerable variation in yield and number of fruits under salinity, particularly in Red Lady.

Exposure of plants to excessive levels of salts results in increased production of reactive oxygen species (ROS) in plants. ROS include the superoxide radical (O^{-}_{2}), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen, which come from endogenous sources as byproducts of normal and essential reactions such as energy generation in mitochondria and detoxification reactions. (Harinasut *et al*, 2003).

Excess levels of ROS are the initiators of a chain reaction that leads to degradation of cellular components. Damage is brought about by the oxidation of photosynthetic pigments, membrane lipids, proteins and nucleic acids by ROS. This state of damage caused by ROS is denoted by the term oxidative stress. One major characteristic of oxidative stress is increased lipid peroxidation wherein the polyunsaturated fatty acids (PUFA) in the plant cells are oxidized. The end product of PUFA oxidation is malondialdehyde (MDA). MDA estimation serves as a measure of the degree of oxidative stress experienced by the tissue (Hodges and Forney 2000).

MDA estimation in cv. Red lady at two levels of stress (Table 2) revealed an increase of 3.5% and 44.8% MDA over the control. This was in accordance with the salt sensitive trait of the cv. Red Lady observed in the field. In contrast, there was no change in MDA content in cv. Tainan subjected to the same degree of stress. Thus, there was practically no oxidative stress in the plants indicative of the salt tolerant trait of the variety. MDA levels of tissues

Table 1. Response of papaya to saline water irrigation

Sl. No.	Parameter	cv. Tainan			cv. Red Lady			C.D. (*P=0.05%)*	CV %
		Control (0.6 dsm^{-1})	Saline treatment (2.0 dsm^{-1})	Saline treatment (4.0 dsm^{-1})	Control (0.6 dsm^{-1})	Saline treatment (2.0 dsm^{-1})	Saline treatment (4.0dsm^{-1})		
1	Yield (tonnes/ha)	60	58	54	55	49	42	2.95	27.0
2	Average fruit weight (kg/fruit)	1.40	1.40	1.30	1.30	1.20	1.15	0.15	14.5
3	Number of fruits	45	44	40	39	37	30	3.90	26.3
4	T.S.S.	12.3	11.3	11.9	13.8	14.0	14.0	0.70	13.5

Table 2. Oxidative stress and antioxidant compounds in two papaya cvs. Red Lady and Tainan, susceptible and tolerant respectively to salinity stress

Sl. No.	Parameter	cv. Tainan			cv. Red Lady		
		Control	T1 (Saliniy 2 dsm^{-1})	T2 salinity 4 dsm^{-1})	Control	T1 (Salinity 2 dsm^{-1})	T2 (salinity 4 dsm^{-1})
1	Oxidative stress/ Malondialdehyde (MDA, in terms of A_{532}/40 mg dry leaf powder)	0.143	0.140	0.145	0.116	0.120 (+3.5%)	0.168 (+44.8%)
2	Total carotenoids (mg/g dry leaf)	65.510	85.96 (+31.2%)	75.59 (+15.4%)	68.920	69.900 (+ 1.3%)	56.280 (-18.3%)
3	Total phenols (mg gallic acid/g dry leaf)	28.930	39.73 (+ 37.3%)	32.07 (+ 10.9%)	28.200	31.270 (+ 10.9%)	21.670 (-23.2%)
4	Total flavonoids (mg catechin/g dry leaf)	6.730	10.14 (+50.7%)	7.59 (+12.8%)	6.910	7.670 (+ 11%)	4.730 (-31.6%)

also served inversely as a measure of cellular membrane integrity (Basra *et al*, 1997).

Plants contain antioxidant compounds which play an important role in detoxifying and regulating levels of ROS. These include carotenoids, ascorbic acid, glutathione, α-tocopherol, phenols and flavonoids (Harinasut *et al*, 2003). Under conditions of various types of stress, plants protect themselves by synthesis of increased levels of various antioxidant compounds (Mandhania *et al*, 2006). Carotenoids are important as antioxidant compounds. They protect chloroplasts against photosensitized oxidation by quenching singlet oxygen, i.e., they function as radical scavengers, effectively binding the ROS and preventing cellular damage (Bosland and Votava, 2000). Results in Table 2 show increased formation of carotenoids (+ 31.2% to 15.4% over control) under conditions of salinity stress in papaya cv. Tainan as compared to cv. Red Lady. The increased carotenoid concentration under of salt stress in Tainan could be a contributory factor for the tolerant trait of the variety. A drought tolerant wheat genotype under water stress, similarly, had the highest carotenoid content (Sairam and Saxena, 2000). Decrease in carotenoid concentration in cv. Red Lady under stress is indicative of increased oxidative stress, possibly contributing to the salt sensitive nature of the cultivar.

Phenolic and flavonoid antioxidants act by free radical scavenging (Subhas Chander and Rao, 2004). Tomato lines having a high level of polyphenols had the most powerful antioxidant potential (Minaggio *et al*, 2003). Results presented in Table 2 show that in papaya cv. Tainan, there was an increase of 37.3% and 10.9% in total phenol content of samples from salt stress of 2 dsm^{-1} and 4 dsm^{-1}, respectively, over the content in unstressed control. In cv. Red Lady, the increase was only 10.9% over control under low salt stress of 2 dsm^{-1}, and, it decreased by 23.2% under high stress of 4 dsm^{-1}. In view of the stress tolerance shown by cv. Tainan under field conditions, increased total phenolic content under stress in this case could be one of the detoxification systems that the plant has developed to limit oxidative damage due to excess formation of ROS, by radical scavenging, which is considered crucial for tolerance (Sarad *et al*, 2004).

Flavonoids are low molecular weight, polyphenolic compounds found in plants. Recent studies provide evidence that accumulation of antioxidant compounds such as flavonoids is one component of a whole set of antioxidant defenses, which help plants to withstand environmental stress (Munne-Bosch, 2005). The cv. Tainan contained 50.7% and 12.8% more flavonoids over control under salt stress of 2.0 dsm^{-1} and 4 dsm^{-1} respectively (Table 2). The corresponding increase in the cv. Red Lady was only +11% over control under 2 dsm^{-1} salt stress and under higher salt stress of 4 dsm^{-1}, the flavonoid content decreased by 31.6%. Thus flavonoids accumulation in the cv. Tainan could be contributing to the salt tolerance by free radical scavenging.

There was 'considerable' to 'substantial' accumulation of sugars in both the cultivars under stress conditions. There were also some sharp differences. Across the varieties there was an accumulation of 26.1% more reducing sugars in 2 dsm^{-1} stressed samples, compared to the control. Also, across varieties, total sugars increased by 12.1% and 10.4% and sucrose increased by 10.2% and 19.5% over the control under 2 dsm^{-1} and 4 dsm^{-1} stress conditions, respectively (Table 3). Thus, salt stress is associated in general with higher sugar levels, more specifically sucrose content.

In cv. Tainan, there was a significant increase of (i) 44.8% and 78% reducing sugars (ii) 15.6% and 45.2% total sugars and (iii) 18.9% and 21.5% sucrose under 2 dsm^{-}

Table 3. Sugar accumulation in salt stressed papaya across varieties

Sl. No.	Treatment	Sugar level (mg/g dry leaf powder)		
		Reducing sugars	Total sugars	Sucrose
1	Control	19.06	37.68	51.76
2	T1 (2 dsm^{-1})	24.04	42.24	57.02
3	T2 (4 dsm^{-1})	14.90	41.58	61.84
4	C.D. (*P=0.05*)	1.93	1.19	4.37
5	CV%	5.76	1.70	4.45

Table 4. Sugar accumulation in salt stressed papaya cvs. Red Lady and Tainan

Sl. No.	Parameter	cv. Tainan			cv. Red Lady			C.D. (*P=0.05*)	CV%
		Control	T1 (Salinity 2 dsm^{-1})	T2 (salinity 4 dsm^{-1})	Control	T1(Salinity 2 dsm^{-1})	T2 (salinity 4 dsm^{-1})		
1	Reducing sugars (mg/g dry leaf)	10.24	14.83	18.23	27.88	33.24	11.57	2.73	5.76
2	Total sugars (mg/g dry leaf)	33.93	39.24	49.25	41.43	45.23	33.91	1.69	1.70
3	Sucrose (mg/g dry leaf)	52.01	61.85	63.18	51.51	52.18	60.51	6.19	4.45

^{1} and 4 dsm^{-1} stress conditions, respectively, over control (Table 4). In contrast, in cv. Red Lady, lesser increase of 19.2% more reducing sugars, 9.2% more total sugars and 1.3% more sucrose over control was observed under 2 dsm^{-1} stress, and, under higher salinity of 4 dsm^{-1}, there was 58.5% decrease in reducing sugars and 18.2% decrease in total sugars, compared to the control. There was a significant increase of 7.8% in sucrose content in cv. Tainan, compared to cv. Red Lady across treatments. CV% values reveal variation in reducing sugar and sucrose content under salinity, both across varieties and between cvs. Tainan and Red Lady.

Thus, there was significant and substantial accumulation of reducing and total sugars and sucrose levels in salt stressed cv. Tainan in contrast to lesser increase or sharp decrease in similarly stressed cv. Red Lady. Thus, cv. Tainan, which resisted salt stress in the field, is associated with increased soluble sugar accumulation. Salt stress resulted in increase in sucrose content in tomato significantly (Ko *et al*, 1999). This has been shown to be due to increased sucrose phosphate synthase (SPS) gene expression under conditions of salt stress. Soluble carbohydrates have a potential role in adaptation to drought and salt stress and, sucrose is believed to be instrumental in maintaining membrane phospholipids in the liquid-crystalline phase and in preventing structural changes in soluble proteins (Kerepesi and Galiba, 2000).

The accumulation of sugars observed is in accordance with the role ascribed to such accumulated solutes in contributing to osmotic adjustment under conditions of stress, leading to maintenance of water uptake and cell turgor and removal of free radicals and stablization of macromolecules, organelles and membranes (Neto *et al*, 2004). Drought and salt tolerant genotypes of wheat accumulated more soluble carbohyderate than did sensitive ones (Kerepesi and Galiba, 2000). Both ionic and nonionic stresses increased the concentration of reducing sugars, sucrose and fructans. Under salt stress conditions, salt tolerant cultivars accumulated soluble carbohydrate fructan, which decreased in salt sensitive cultivars. Kerepesi and Galiba (2000) conclude that water soluble carbohydrates content could be a useful marker for selecting genotypes that are more drought or salt-tolerant. The reducing and total sugar and sucrose content in papaya cultivars Red Lady and Tainan show the same trend (Table 4). As in the case of wheat, soluble sugar content increased in the tolerant cv. Tainan, as against much lesser increase and very considerable decrease in the sensitive cv. Red Lady. Thus, this information on soluble sugar accumulation could be useful as a breeding tool for selecting salt-tolerant papaya genotypes.

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Studies on physical and chemical characteristics of pomegranate cultivars in Kashmir valley

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ABSTRACT

Ten pomegranate (*Punica granatum* L.) cultivars, namely, Kabuli Kandhari, Chawla, Ganesh, Mridula, Jyoti, G-137, Dholka, Bedana, Kandhari and Local Check were evaluated for different physical and chemical characteristics of fruit at the Central Institute of Temperate Horticulture, Srinagar, during 2004. Fruit weight, diameter and volume was significantly higher in cv. Bedana compared to the rest of the cultivars. Cultivar Kandhari recorded significantly less rind thickness when compared to other cultivars. Cultivar Chawla exhibited less cracking per cent followed by Kandhari. Total soluble solids and total sugars were highest in cv. Kandhari whereas less acidity was recorded in cvs. Ganesh and G-137% acidity was lowest in cv. G-137 (0.41) and highest in cv. Bedana (0.81). Highest ascorbic acid content was found in cv. Kabuli Kandhari. The highest anthocyanin content was observed in cv. Ganesh and lowest in cv. Chawla. Juice content was found to be maximum in Bedana. The lowest anar butterfly attack was observed in cv. Bedana. The data revealed overall superior performance of cv. Bedana and Kandhari with regard to physical and chemical characteristics and these can be recommended for commercial cultivation in the Karewa belt of Kashmir valley.

Key words: Pomegranate, physical and chemical characteristics of fruit

INTRODUCTION

Pomegranate (*Punica granatum* L.) fruit deserves special attention of consumers interested in nutritional food with excellent taste. Dietary supplementation with pomegranate is believed to relate with cancer prevention (Afaq *et al*, 2003). The tree is deciduous in low winter-temperature areas but, in tropical and subtropical areas, it is evergreen or partially deciduous. High-quality fruits can be produced where there are cool winters and hot, dry summers. It enjoys reputation for its healthy, dietetic and medicinal properties. The fruit is now gaining importance in temperate regions due to its hardy nature and capacity to tolerate drought, frost and alkaline conditions. In spite of the economic importance of pomegranate, information on its physico- chemical composition is under temperate conditions meagre and, therefore, the present investigation was undertaken to evaluate important cultivars for their physical and chemical characteristics under the temperate conditions of Kashmir Valley.

MATERIAL AND METHODS

The investigation was conducted at the Central Institute of Temperate Horticulture, Srinagar, in 2004. Ten pomegranate cultivars of five years age having uniform vigour were evaluated in a randomized block design replicated thrice. Five plants per replication in each cultivar were taken randomly for recording data. The plants were raised under uniform cultural practices. Fruits were harvested when most of them were red in colour and were transferred to the laboratory to sort for size and uniformity of shape. Fruit shape, colour and general appearance was recorded on a hedonic scale. The chemical constituents of the edible portion were estimated as per methods detailed in A.O.A.C. (1984). The TSS of fruit juice was estimated with a hand-refractometer. Anthocyanin content was estimated as per Ranganna (1986).

RESULTS AND DISCUSSION

Most of the physical characters studied showed significant difference among cultivars (Table 1). Cultivar Bedana had the maximum fruit weight, diameter and volume (232.12g, 7.68 cm and 237.62 cm^3, respectively), whereas, lowest values in these parameters were recorded in cv. Ganesh. Local check recorded an average 188.12 g of fruit

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Table 1. Physical characteristics of different pomegranate cultivars

Cultivar	Fruit weight	Fruit diameter (g)	Fruit volume (cm)	Specific gravity (cm^3)	Rind thick-ness (mm)	Rind weight (g)	Number of seeds $fruit^{-1}$	Aril weight (g)	Cracking (%)	Fruit shape	Fruit colour	General appea -rance	Anar butterfly incidence (%)
Kabuli Kandhari	174.36	6.86	169.45	0.985	4.22	71.37	482.09	0.213	19.24	4.00	3.93	3.30	12.49
Chawla	166.91	6.63	152.74	1.036	4.95	54.62	438.16	0.256	06.32	2.73	3.33	2.84	10.09
Ganesh	110.28	5.76	100.28	0.950	3.10	52.52	275.88	0.210	26.30	3.92	4.00	2.80	08.33
Mridula	143.06	6.36	134.97	0.973	3.24	50.41	426.62	0.216	19.50	3.80	2.46	2.35	12.38
Jyoti	162.36	6.50	144.31	0.986	3.91	57.17	449.14	0.233	25.17	2.40	0.46	1.50	10.25
G-137	189.49	7.09	187.21	0.971	3.41	75.15	423.00	0.270	31.40	1.73	2.40	2.48	08.51
Dholka	216.61	7.38	211.13	0.965	3.58	70.73	468.33	0.316	19.54	3.93	2.60	3.50	09.94
Bedana	232.12	7.68	237.62	0.966	4.13	73.75	546.94	0.289	18.15	4.00	2.73	3.57	08.36
Kandhari	222.88	7.49	220.69	0.956	2.92	69.36	502.99	0.305	16.52	4.00	2.66	3.44	11.26
Local Check	188.06	7.06	184.59	0.990	4.15	74.45	486.33	0.233	21.20	3.06	1.80	2.50	11.42
SE d	9.35	0.06	4.13	0.015	0.25	4.73	12.60	0.008	0.78	0.17	0.26	0.17	0.61
CD *(P=0.05)*)	19.66	0.11	8.68	0.03	0.54	9.95	26.48	0.017	1.64	0.36	0.55	0.36	1.30
CV (%)	6.34	0.98	2.90	1.94	8.45	8.93	3.43	4.03	4.71	6.40	12.23	7.59	7.36

weight $plant^{-1}$ and was significantly superior to five but not cultivars cvs. Bedana, Kandhari, Dholka and G-137. Variation in fruit weight and diameter was in accordance with findings of Bist *et al* (1994). The minor deviation with respect to fruit weight may be due to variations in the form, as, sometimes they are obscurely ridged and many-sided, as reported by Nath and Randhawa (1959). Mmaximum specific gravity was recorded in cv. Chawla (1.036) followed by Local Check (0.990) and Jyoti (0.986). Lowest specific gravity was exhibited by cv. Ganesh (0.950). Generally, fruit weight, diameter and volume are directly proportional to each other. Increase in fruit size, volume and weight and decrease in specific gravity was also reported by Dhillon and Kumar (2004) and Khodade *et al* (1990). It is obvious from the data that lowest rind-thickness was observed in cv. Kandhari (2.92 mm) which was significantly less compared to rest of the cultivars under test. Higher rind-thickness was recorded in cv. Chawla (4.95 mm). Rind weight is also an important factor in pomegranate as it constitutes the non- edible part of the fruit. The lowest rind-weight was registered in cv. Mridula (50.41 g $fruit^{-1}$), followed by cvs. Chawla and Jyoti. These results are in close conformation with findings of Bist *et al* (1994) and Misra *et al* (1983).

It is evident from the data that cv. Bedana recorded maximum number of seeds/ fruit (546.94), followed by Kandhari (502.99) and Local Check (486.33). The latter two cultivars were statistically at par with each other. The results obtained are in agreement with findings of Misra *et al* (1983). As regards aril-weight, cv. Dholka (0.316 g) was significantly superior to the rest of the cultivars. The least aril-weight was recorded in cv. Ganesh (0.210 g), followed by Kabuli Kandhari (0.213 g) and Mridula (0.216 g). Increase in aril-weight with advancement of maturity in Cv. Kandhari was also observed by Dhillon and Kumar (2004). Maximum cracking was registered in cv. G-137 (31.40%), followed by Ganesh (26.30%) and Jyoti (25.17%). Lowest cracking incidence was observed in cv. Chawla (6.32%). Variability in this trait is attributed to the fact that some fruits may have higher rind-thickness due to which these do not crack easily. Secondly, variation in cracking may be also due to a sudden change in the climate at the time of maturity, besides variable moisture and tolerance of cultivars to cracking (Bankar and Prasad, 1992). The results are also supported by the findings of Shulman *et al* (1984).

Analysis of variance (ANOVA) revealed that highest scoring index for fruit shape in cvs. Bedana and Kandhari (4.00 points each), followed by Dholka (3.93 points) and Ganesh (3.92 points). The lowest scoring index was noticed in cv. G-137 (1.73 points). The highest fruit colour value was recorded in cv. Kandhari (4.00 points), followed by Bedana and Kabuli Kandhari. These cultivars were, however, statistically at par but significantly superior to rest of the cultivars. Regarding the general appearance of the fruit, highest scoring index was observed in cv. Bedana (3.57), followed by cvs. Dholka (3.50) and Kandhari (3.44) Lowest scoring index was observed in cv. Jyoti (1.50)

Table 2. Chemical composition of different pomegranate cultivars

Cultivar	Total soluble solids (%)	Total sugars (%)	Acidity (%)	TSS/ Acid ratio	Ascorbic acid (mg 100^{-1} ml)	Anthocyanin (mg 100^{-1} g)	Juice content (%)
Kabuli Kandhari	15.46	8.16	0.64	24.20	13.26	19.37	45.88
Chawla	13.56	7.81	0.45	30.79	09.40	10.34	49.72
Ganesh	14.42	8.19	0.43	33.84	12.94	20.30	41.71
Mridula	15.61	8.56	0.76	20.58	13.10	15.35	46.13
Jyoti	14.03	8.50	0.44	32.03	12.15	11.24	47.42
G-137	15.49	8.33	0.41	38.12	11.31	13.21	50.39
Dholka	15.55	8.38	0.52	30.13	10.65	14.42	50.55
Bedana	15.77	9.62	0.81	19.48	13.36	16.27	50.83
Kandhari	15.74	9.75	0.57	27.69	10.33	18.34	49.80
Local Check	13.85	8.05	0.47	29.70	9.76	14.18	48.92
SE d	0.28	0.19	0.021	2.13	0.74	1.13	1.07
CD *(P=0.05)*)	0.60	0.40	0.04	4.48	1.56	2.38	2.25
CV (%)	2.35	2.74	4.73	9.11	7.86	9.09	2.73

which was inferior to even Local Check (2.50). The findings revealed that cvs. Bedana, Dholka, Kandhari, Kabuli Kandhari and Chawla were best with regard to these traits. As far as anar butterfly incidence is concerned, it was higher in cv. Kabuli kandhari (12.49%) and lower in cv. Ganesh (8.33%). This difference in anar butterfly incidence in the cultivars may be due to variable biological behavior of the cultivars and their inherent capacity to tolerate the incidence.

The TSS of the juice in different cultivars ranged from 13.56 (cv. Chawla) to 15.77^0 Brix (cv. Bedana). However, cvs. Bedana, Kandhari, Mridula, Dholka and Kabuli Kandhari were statistically at par. The findings are in conformity with that reported by Parmar and Kakushal (1982) and Bist *et al* (1994). The highest total sugars were registered in cv. Kandhari (9.75%), followed by cvs. Bedana (9.62%) and Mridula (8.56%). The lowest sugar content was recorded in cv. Chawla (7.81%). Results obtained in the present study are in accordance with findings of Malhotra *et al* (1983) and Jagtap *et al* (1992). Fruit acidity ranged from 0.41 (cv. G-137) to 0.81% (cv. Bedana). Intervarietal differences were highly significant. Increase in TSS and decrease in acidity during fruit development was in accordance with findings of Kumar and Purohit (1989).

The total soluble solids/ acid ratio ranged from 19.48 (cv. Bedana) to 38.12 (cv. G-137). The cultivar G-137 was significantly superior to the rest of the cultivars. As far as ascorbic acid is concerned, cv. Bedana, at par with cvs. Kabuli Kandhari and Mridula, recorded the highest ascorbic acid content of 13.36, 13.26 and 13.10 mg/ 100ml, respectively compared to the rest of the cultivars. Lower ascorbic acid content was observed in cv. Chawla (9.40 mg/ 100 ml). The variation in ascorbic acid content has also been reported by Malhotra *et al* (1983) and Jagtap *et al* (1992) in pomegranate. Cultivar Ganesh registered the highest anthocyanin content (20.30 mg/ 100 g), followed by cv. Kabuli Kandhari (19.37 mg/ 100g) and cv. Kandhari (18.34 mg/ 100 g), whereas, the lowest anthocyanin content was recorded in cv. Chawla (10.34 mg/ 100g). The variation in anthocyanin content among cultivars is attributed to genetic make up of the plant. Significant varietal difference was also reported by Legua *et al* (2000). The juice percentage was significantly higher in cv. Bedana (50.83%), Dholka and G-137 compared to the other cultivars. Siddappa (1943) also reported that cultivars differed in their juice content due to differences in their genetic constitution. From the present study it can be concluded that cvs. Kandhari, Bedana and Dholka are superior in physico-chemical characteristics and may be recommend for commercial cultivation under Srinagar conditions and can be used for further improvement of the crop.

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Effect of dry and wet storage on post harvest life and flower quality in cut tulip cv. Cassini

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ABSTRACT

Experiments were conducted during 2002-03 and 2003-04 to study the influence of storage methods and duration on post harvest quality of cut tulip cv. Cassini. Cut tulips cv. Cassini stored either dry or wet at 4^0C for 0,2,4,6 and 8 days showed that days to flower opening was the lowest in those kept under wet storage for 6 and 8 days. Flower opening was better with 0.2 and 4 days of dry or wet storage whereas flowers stored dry for 8 days did not open at all. Flower size and vase life decreased with the increase in storage period. Larger flowers were obtained with dry and wet storage of 0 and 2 days whereas higher vase life was obtained with zero days of wet and dry storage and 4 and 6 days of wet storage.

Key words: Tulip, storage, vase life

INTRODUCTION

Tulips are hardy spring flowering bulbs with most stems terminating into a single flower which has six petals (Anonymous, 2001-2002) and represents the largest geophyte crop worldwide. It has gained popularity owing to its beauty and economic value. The use of tulips vary from cut flowers, formal plantings in borders and flower beds, indoor forcing and planting on the rock gardens. Tulips have tremendous potential both in the international and domestic markets (Desh Raj, 1999). However, the quality of cut tulips production are known to be influenced by both pre and post-harvest practices. Post harvest losses can be reduced by suitable pre and post harvest management practices. Information on the quality of clones of field grown cut tulip blooms at room temperatures following low temperature dry storage is essential for profitable storage and marketing of tulip blooms (New, 1964). Since the information available on storage of cut tulips in scanty, the present investigation was undertaken with the objective of finding out suitable storage duration for cut tulips.

MATERIAL AND METHODS

Healthy and blemish-free scapes were cut, pre-cooled in a refrigerator and were divided into two lots. The scapes were weighed and stored at 4^0C. One lot of scapes was kept in large beakers with their base dipped in distilled water and the other lot was bunched and stored dry at 4^0C. The control scapes were placed directly in distilled water for observations. Scapes were taken out from both the lots after 2, 4, 6 and 8 days of storage and placed in the distilled water for vase life studies. The observations on vase life were recorded as per the procedure given by Venketarayappa *et al.,* (1980).

Days taken to flower opening: Data of flower opening was recorded and then days calculated from the date of placing in the distilled water in vase.

Fresh weight changes (% of initial weight): The difference between the weight of flask solution + scape weight of flask + solution represented the fresh weight (g) of the scape on that particular date.

Fw= (C+S+F)- (C+S)

Where: Fw = Fresh weight
C = Container (flask)
S = Solution
F = Scape

After this the per cent fresh weight change was calculated by the formula:

$$\text{Fresh weight change (\%)} = \frac{\text{F.W of a particular day- initial fresh weight}}{\text{Initial fresh weight}} \times 100$$

Water uptake (g/scape): The difference between consecutive measurement of the flask + solution (without scape) represented the water uptake:

$W_U = \{C+S\}_1 - \{C+S\}_2$

Where W_U = water uptake

Water loss (g/scape) transpirational g/scape: The difference between consecutive measurements of flask + solution + flower scape represented the water loss.

W_1 (transpirational loss) = $\{C+S+F\}_1 - \{C+S+F\}_2$

Where W_1 = water loss

Water balance (g/scape): Water uptake minus transpirational loss of water represented water balance:

$W_U = W_U - W_1$

Where W_B = Water balance

Water loss/ water uptake ratio: Transpirational loss of water divided uptake represented the water loss/ water uptake ratio:

$$\text{Ratio} = \frac{W_1}{W_U}$$

Flower opening (%): Number of flowers that opened fully in the vase was counted and then per cent flower opening counted out of the total flowers placed in the containers.

Flower diameter (cm): Flower diameter was taken across the fully opened flowers.

Vase life (days): Number of days was counted from the date of opening till the tepals lost their decorative value.

RESULTS AND DISCUSSION

In general, number of days taken to flower opening decreased with the increase in storage period either in dry or wet storage. During first year significantly maximum days (7.0) to flower opening were taken by zero day storage in water which was at par with 0 and 2 days of dry storage (6.44 and 6.11, respectively). Cut scapes stored in water for 6 and 8 days took minimum days of 3.66 each for flower opening whereas tulip flowers stored dry for 8 days did not open at all. Similar trend was followed during the second year also (Table 1).

During both the years of study cut tulips stored in water for 8 days gave minimum flower opening percentage (54.73 and 48.24, respectively.) Whereas, significantly maximum flower opening was recorded with scapes stored for 0,2 and 4 days of dry and wet storage.

Aekyung *et al* (1996) reported that when cut lilium flowers were treated with certain preservatives before storage at 3 or 6 ^oC for 1-5 days, they failed to open after storage for 5 days or showed rolling of petals and sepal edges. In Narcissus cut flowers stored either dry or wet for 14 days at 1-2 ^oC at >90 per cent RH, some flowers failed to open when transferred to ambient temperatures (Nicholas and Wallis, 1972; Rees, 1985).

Flower diameter also exhibited decreasing trend with the increase in dry or wet storage (Table1). During both the years larger flowers (6.90 and 7.0 cm, respectively) were obtained with zero day dry storage which was at par with zero day of wet storage (6.36 and 6.61 cm, respectively). Flower scapes stored dry for 6 days and wet for 8 days were at par with each other in recording the smaller flowers of 5.52 and 5.62 cm, respectively, during first year and 5.54 and 5.40 cm during second year. Wallis (1968) reported that increased storage duration reduced flower diameter in cut Narcissus. Katwata *et al* (1995) reported that size of the second floret of *Polianthes tuberosa* decreased with the increase in storage from 24-72 h at 4^oC.

Daily water uptake, water loss and water balance of cut tulips did not follow any general trend because all the treatments were not placed in vase on a single day.

Pooled data of two years revealed (Table 2) that on day 8, when all the treatments were in vase, maximum water uptake was recorded by zero day wet and dry stored samples (3.73 and 3.29 g/ scape, respectively) and minimum water uptake (1.47 g/scape) by 2 day dry stored samples Song *et al* (1992) reported that water uptake of cut roses cv. Sonia decreased with increased in length of dry storage. Song *et al* (1995) further reported that solution uptake decreased with the increase in storage duration of cut hybrid delphinium.

On day 8 and 10, maximum water loss was (Table 2) recorded by zero day in dry storage (3.59 and 3.38 g/ scape, respectively). Minimum water loss on day 8 was observed in scapes stored in water for 4 days (1.66 g/ scape) and on day 10 in scapes stored dry for 8 days (1.44 g/scape). The cut tulips did not open at all under later treatment and water loss was less owing to less surface available for transpirational loss. As per Sanket *et al* (1994) water loss slowed in cut Anthurium as the storage temperatures decreased.

Treatments exhibited negligible variation as regards water balance upto 6 days of storage whether dry or wet but on 8th and 10th day many treatments showed negative water balance. On day 8, lowest negative water balance (-0.60 g/ scape) was recorded by 4 days of dry storage and highest positive water balance was recorded by 6 days in dry storage

(0.50 g/ scape). Sanket *et al* (1994) reported that all the components of water balance declined rapidly at all storage temperatures for first 5 days when cut Anthuriums were held for 30 days at 8, 13, 18 and 28^{0}C (Table 2).

The trend depicted (Table 1) that vase life of cut tulips decreased with the increase in storage period. During both the years, significantly maximum vase life of 7.55 and 7.99 days, respectively was recorded with cut scapes when

Table 1. Effect of dry and wet storage on vase life studies of cut tulips (2002-04)

Treatments	Days to flower opening			Flower diameter(cm)			Vase life(day)			Flower opening(%)		
	1a	Highly significant	Mean	I	II	Mean	I	II	Mean	I	II	Mean
Dry storage (days)												
(0)	6.44	6.77	6.60	6.90	7.0	6.95	7.21	7.66	7.43	100.00 (90.00)**	88.89 (78.24)	94.44 (84.12)
(2)	6.11	6.55	6.33	6.71	6.30	6.50	7.10	6.77	6.93	100.00 (90.00)	88.89 (78.24)	94.44 (84.12)
(4)	6.11	6.44	6.27	5.59	5.58	5.58	6.10	5.70	5.60	88.89 (78.24)	77.77 (66.48)	83.33 (72.36)
(6)	4.88	4.11	4.49	5.52	5.54	5.53	4.74	4.99	4.86	77.77 (66.48)	66.66 (54.73)	72.21 (60.60)
(8)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (-0.00)	0.00 (-0.00)	0.00 (-0.00)
Wet storage (days)												
(0)	7.0	6.88	6.94	6.36	6.61	6.48	7.55	7.99	7.78	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
(2)	5.66	5.33	5.49	6.13	6.38	6.25	7.44	7.88	7.66	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
(4)	4.55	4.44	4.49	5.91	6.19	6.05	6.88	6.44	6.66	100.00 (90.00)	88.89 (78.24)	94.44 (84.12)
(6)	3.66	4.00	3.83	5.80	4.48	5.64	4.99	5.33	5.16	77.77 (66.48)	66.66 (54.73)	72.21 (60.60)
(8)	3.66	3.88	3.77	5.62	5.40	5.51	4.66	4.22	4.44	66.66 (54.73)	55.55 (48.24)	61.10 (51.48)
CD *(P=0.05)*	2.50	2.22	-	0.86	1.33	-	0.73	1.94	-	18.99	26.44	-

a Year 2002-03

b Year 2003-04 * Data in parenthesis are the arc sin transformed values.

Table 2. Effect of dry and wet storage on daily water uptake , water loss and water balance (g/scape) of cut tulips cv. *Cassini* (Pooled data of two years).

Treatments	Days in vase																	
	0			2			4			6			8			10		
	WU	WL	WB	WU	WL	WB	WU	WL	WB	WU	WL	WB	WU	WL	WB	WU	WL	WB
Dry storage (days)																		
(0)	5.57	3.60	1.96	4.17	2.69	1.48	3.59	3.31	0.29	3.01	3.70	0.81	3.29	3.59	-0.29	2.57	3.38	-0.80
(2)	-	-	-	3.58	1.85	1.73	3.01	2.07	0.94	2.13	1.88	0.24	1.47	1.89	-0.42	1.28	1.47	-0.14
(4)	-	-	-	-	-	-	3.96	9.48	0.98	2.36	1.22	1.26	2.20	2.70	-0.60	1.49	2.20	-0.63
(6)	-	-	-	-	-	-	-	-	-	2.97	1.63	1.34	2.28	1.43	0.84	1.97	1.46	0.50
(8)	-	-	-	-	-	-	-	-	-	-	-	-	2.20	1.24	0.97	1.43	1.44	0.31
Wet storage (days)																		
(0)	4.67	3.24	1.49	3.85	2.86	0.99	3.49	2.96	0.69	2.12	1.32	0.96	3.73	2.82	0.90	1.73	2.76	-1.01
(2)	-	-	-	4.33	3.29	2.20	3.56	3.06	0.49	3.11	3.45	-0.33	1.93	2.43	-0.16	1.51	2.53	-1.02
(4)	-	-	-	-	-	-	4.77	3.63	1.40	3.22	2.83	0.67	2.29	1.66	0.63	2.10	3.32	-0.87
(6)	-	-	-	-	-	-	-	-	-	4.85	3.44	1.40	2.45	2.33	0.31	3.13	2.66	0.47
(8)	-	-	-	-	-	-	-	-	-	-	-	-	2.0	1.79	0.21	2.47	2.35	0.12
CD *(P=0.05)*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS: Non-significant; WU: Water uptakeWL: Water lossWB: Water balance

Table 3. Effect of dry and wet storage on fresh weight changes (%) of cut tulips scapes in vase (pooled data of two years)

Treatments	Days in vase					
	0	2	4	6	8	10
Dry storage (days)						
(0)	14.71	15.31	25.66	28.65	33.05	32.67
		(22.32)*	(30.13)	(31. 79)	(34.88)	(34.22)
(2)	10.59	4.05	18.51	26.08	28.51	25.52
		(10.86)	(24.88)	(30.51)	(32.22)	(30.17)
(4)	12.63	-	9.17	13.63	18.84	20.98
			(16.66)	(20.91)	(25.60)	(26.78)
(6)	12.14	-	-	7.16	11.51	14.00
				(14.39)	(18.80)	(19.00)
(8)	08.84	-	-	-	14.08	18.10
					(20.73)	(23.09)
Wet storage (days)						
(0)	11.11	19.24	38.75	45.22	41.07	41.70
		(25.07)	(38.39)	(42.84)	(39.61)	(40.05)
(2)	11.96	14.74	23.56	28.32	22.35	22.79
		(21.11)	(28.42)	(31.61)	(27.16)	(26.22)
(4)	12.55	-	15.26	20.29	35.92	29.84
			(21.65)	(26.00)	(35.99)	(32.98)
(6)	11.73	-	-	27.84	34.58	36.39
				(29.08)	(35.37)	(36.81)
(8)	11.98	-	-	-	37.01	39.86
					(36.31)	(38.33)
CD *(P=0.05)*	NS	9.52	13.03	12.80	11.28	13.05

NS : Non-significant

* Data in parentheses are the arc sin transformed values.

stored wet for zero day. Minimum vase life of 4.66 and 4.22 days was recorded with wet storage for 8 days whereas flowers did not open when tulip cut scapes were dry stored for 8 days. Swart (1986) reported that a long period of dry storage (3 days at 2 ^{o}C) had an adverse effect on vase life of cut tulips but storing cut flowers by placing them in water prevented these negative effects. Vase life of tulips decreased as the storage temperature increased (Doss, 1986) and longer periods of storage were possible at 1.10 ^{o}C than at 4-5 or 10 ^{o}C. Mor *et al.* (1989) also reported that vase life of roses cv. Gabriella stored at 1^{o}C for 3 weeks was less than vase life of fresh flowers.

Changes in fresh weight were influenced significantly by dry and wet storage (Table-3) throughout the period of study though all treatments were not placed in vase on one single day. The general trend revealed that tulip scapes gained weight upto 8 days of observation, thereafter, some of the treatments showed decrease in fresh weight. Swart (1991) reported that flowers stored in water showed an increase in fresh weight. After all storage period, dry stored flowers showed increase in fresh weight upto day three thereafter, it decreased and the decrease in fresh weight corresponded with a visual decline in flower quality.

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Effect of date of harvest and floral preservatives on vase life of cut flowers in tuberose (*Polyanthes tuberosa* L.) cv. Double

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ABSTRACT

Studies conducted to find out the effect of date of harvesting and floral preservatives on vase life and quality of tuberose cv. Double revealed that among treatments, harvesting on 1st October (D_8) was better for longer vase life, whereas, 15th August (D_5) for minimum loss of water, maximum fresh weight of the spike and percentage of opened florets. Similarly, harvesting on 15th September (D_7) was found better for longest floret longevity as well as loss uptake ratio. In case of floral preservatives, the treatment 500 ppm aluminum sulphate + 4% sucrose (C_6) was found better for longer vase life, maximum uptake of water, lowest loss-uptake ratio and maximum fresh weight of spike, whereas, 400 ppm 8-HQS + 4% sucrose (C_8) for maximum floret longevity and floret circumference as well as maximum percentage of opened and lowest percentage of neck bent florets. The treatment, 50 ppm silver nitrate + 4% sucrose (C_3) exhibited lowest loss of water. In case of interaction effect, 1st October with 500 ppm aluminum sulphate + 4% sucrose (D_8C_6) was found superior for maximum vase life of spike, highest uptake of water and fresh weight of spike.

Key words : Tuberose, vase life, floral preservatives

INTRODUCTION

Tuberose is grown on a wide range of soil and climatic conditions but it flowers best in warm and humid climate. Among four types of tuberose, the Double floret type is mainly cultivated for cut flowers, whereas single types are grown for loose flower production and also for extraction of essential oil. The post harvest management is one of the most important factors in the production and marketing of cut flowers. At present flower growers are not aware of standardized post harvest technology including the harvesting time and use of floral preservatives to extend the vase life. Available literature indicated the meager work done on date of harvesting and hence an attempt is made to standardize the date of harvesting and use of floral preservatives in tuberose (*Polianthes tuberosa* L.) cv. Double to extend the vase life of cut flower during rainy season.

MATERIAL AND METHODS

The present investigation was conducted at Department of Horticulture, College of Agriculture, Junagadh Agricultural University, Junagadh (Gujarat) during rainy season of the year 2003 and 2004 in the factorial C R D. The treatments comprised of different floral preservatives like T_1- sucrose @ 4%, T_2- Aluminum sulphate @ 500 ppm, T_3-Silver nitrate @ 50 ppm, T_4-8-HQS @ 400 ppm, T_5- citric acid @ 300 ppm and their combinations with sucrose @ 4% (T_6, T_7, T_8, T_9) and T_{10}- Distilled water (Control). The trial was repeated at fortnightly interval during the season with each of the 8 dates of harvesting (D_1 - 15th June, D_2 -1st July, D_3 -15th July, D_4 - 1st August, D_5 -15th August, D_6 - 1st September, D_7 - 15th September, D_8 -1st October) starting from 15th June, 2003 to 1st October, 2003. The same was repeated for second year during 2004. Observation on mean temperature, relative humidity and evapo-transpiration rate were recorded. Healthy, uniform and homogenous spikes were selected and harvested at one or two floret opening stage. Spikes were made to uniform length through trimming. Observations like uptake of water, water loss, loss-uptake ratio, fresh weight of spike, percentage of opened, partial opened, neck bent and abscised florets as well as floret longevity, floret circumference and vase life of the spikes were recorded.

Table 1. Effect of date of harvesting and floral preservatives on vase life of spike, floret longevity and circumference of tuberose.

Treatments	Vase life (Days)			Floret longevity (Days)			Floret circumference (cm)		
	2003	2004	Pooled	2003	2004	Pooled	2003	2004	Pooled
					Date of harvesting				
D_1	11.97	12.65	12.31	3.55	3.63	3.59	6.78	6.84	6.81
D_2	11.72	11.87	11.80	4.16	4.16	4.16	7.26	6.85	7.06
D_3	12.84	10.87	11.86	3.87	3.81	3.84	6.59	7.09	6.84
D_4	11.30	10.52	10.91	4.09	3.86	3.97	6.27	6.09	6.18
D_5	12.55	13.96	13.25	3.87	3.87	3.87	5.36	6.08	5.72
D_6	10.97	10.30	10.63	4.22	4.67	4.45	6.70	6.71	6.71
D_7	12.80	15.03	13.92	3.81	4.12	3.96	6.59	6.72	6.65
D_8	14.25	14.43	14.34	3.86	4.16	4.01	6.42	6.56	6.49
S.Em.±	0.108	0.123	0.66	0.021	0.037	0.11	0.063	0.063	0.18
C.D. *(P=0.05)*	0.30	0.34	2.20	0.06	0.10	0.38	0.18	0.18	0.59
					Floral preservatives				
C_1	13.12	13.61	13.36	3.78	3.94	3.86	6.11	5.85	5.98
C_2	13.09	13.26	13.17	4.14	4.65	4.39	6.77	6.64	6.70
C_3	10.90	11.40	11.15	3.78	3.61	3.70	5.84	5.57	5.70
C_4	12.25	12.16	12.20	4.39	4.05	4.22	7.45	7.86	7.65
C_5	12.15	12.56	12.36	3.62	3.96	3.79	6.21	6.67	6.44
C_6	14.37	14.50	14.44	4.37	4.56	4.46	6.92	7.28	7.10
C_7	11.96	12.06	12.01	3.60	3.66	3.63	5.85	5.67	5.76
C_8	12.72	12.37	12.54	4.44	4.49	4.46	7.84	7.99	7.91
C_9	12.92	12.51	12.71	3.73	3.89	3.81	6.39	7.03	6.71
C_{10}	9.52	10.13	9.82	3.42	3.55	3.49	5.60	5.63	5.62
S.Em.±	0.121	0.137	0.18	0.023	0.041	0.12	0.070	0.070	0.17
C.D. *(P=0.05)*	0.34	0.38	0.56	0.06	0.12	0.38	0.20	0.20	0.53
					Interaction D x C				
S.Em.±	0.34	0.39	0.55	0.07	0.12	0.19	0.20	0.20	0.29
C.D.*(P=0.05)*	0.96	1.09	1.55	0.18	0.33	0.55	0.55	0.56	NS

RESULTS AND DISCUSSION

Vase life of spike

Maximum vase life of spike (14.34 days) was observed at 1st October (D_8) date of harvesting, whereas, among preservatives, highest (14.44 days) was recorded in 500 ppm aluminum sulphate+ 4% sucrose (C_6) (Table 1). The interaction was also found to be significant with their combination (D_8C_6). Similarly minimum vase life (10.63 and 9.82 days) was noted at D_6 (1st September) and under control (C_{10}), respectively, as well as in their interaction (D_6C_{10}). The extended vase life might be due to decreased loss of water as well as loss-uptake ratio, tends to increase the water balance in the spike because of lower range of temperature and evapo transpiration with higher range humidity.

Aluminum sulphate is responsible for lowering the pH of petal and acidifying the holding water, this might have reduced the bacterial growth and improved water uptake. It also reduces transpiration by inducing the stomatal closure. Exogenous sucrose serves as source of energy and respiratory substrate for the maintenance of osmotic potential in flowers. The translocated sucrose accumulates in the flowers increasing its osmotic concentration which improves the ability of the tissue to absorb water and maintain turgidity. Similar results were also reported by Gowda (1990) and Reddy and Singh (1996) in tuberose.

Floret longevity and circumference

Maximum floret longevity (4.45 days) was recorded in spikes harvested on 1st September (D_6). Among floral preservatives, highest floret longevity (4.46 days) was recorded in the C_8 treatment (400 ppm 8-HQS + 4% sucrose) (Table 1).

Highest floret circumference (7.06 cm) was registered in 1st July (D_2) harvested spikes and the treatment 400 ppm 8-HQS + 4% sucrose (C_8) recorded (7.91 cm). The interaction effect was significant for floret longevity, but not for circumference.

Uptake of water, loss of water and water loss-uptake ratio

The uptake of water, loss of water and water loss-uptake ratio were significant (Table 2) and recorded the best values (83.10 g, 46.85 g and 1.53) in spikes harvested on 15th June (D_1), 15th August (D_5) and 1st October (D_8),

Table 2. Effect of date of harvesting and floral preservatives on uptake of water, loss of water and loss-uptake ratio during vase life of tuberose.

Treatments	Uptake of water (g)			Loss of Water (g)			Loss-uptake ratio		
	2003	2004	Pooled	2003	2004	Pooled	2003	2004	Pooled
					Date of harvesting				
D_1	84.53	81.67	83.10	137.23	124.87	131.05	1.68	1.58	1.63
D_2	39.90	69.27	54.58	71.17	114.43	92.80	1.91	1.73	1.82
D_3	34.97	39.70	37.33	59.03	71.03	65.03	1.74	1.97	1.86
D_4	25.43	25.87	25.65	61.33	60.50	60.92	2.6	2.57	2.58
D_5	26.93	27.27	27.10	38.20	55.50	46.85	1.56	2.22	1.89
D_6	44.67	31.17	37.92	77.70	59.07	68.38	1.77	1.98	1.87
D_7	53.03	71.33	62.18	104.80	105.33	105.07	2.15	2.15	2.15
D_8	74.50	86.93	80.72	111.60	125.40	118.50	1.57	1.48	1.53
S.Em.±	0.344	0.695	6.71	0.594	0.911	9.69	0.029	0.041	0.14
C.D. *(P=0.05)*	0.96	1.95	22.41	1.66	2.55	32.33	0.08	0.12	0.46
					Floral preservatives				
C_1	53.62	61.38	57.50	80.88	92.92	86.90	1.52	1.63	1.57
C_2	64.50	72.13	68.31	93.33	104.63	98.98	1.66	1.9	1.78
C_3	36.79	37.17	36.98	72.17	72.63	72.40	2.18	2.42	2.3
C_4	45.42	47.38	46.40	87.63	88.58	88.10	1.98	2.14	2.06
C_5	36.67	52.58	44.63	73.13	85.75	79.44	2.05	1.87	1.96
C_6	72.13	82.71	77.42	89.58	109.21	99.40	1.35	1.48	1.42
C_7	37.75	40.50	39.13	75.54	69.96	72.75	2.1	1.94	2.02
C_8	47.38	52.63	50.00	96.58	99.33	97.96	2.12	2.18	2.15
C_9	47.50	51.67	49.58	81.29	86.75	84.02	1.76	1.9	1.83
C_{10}	38.21	43.38	40.79	76.21	85.42	80.81	2.02	2.14	2.08
S.Em.±	0.385	0.777	2.29	0.664	1.018	3.72	0.032	0.046	0.07
C.D. *(P=0.05)*	1.08	2.18	7.30	1.86	2.85	11.88	0.09	0.13	0.24
					Interaction D x C				
S.Em.±	1.09	2.20	5.67	1.88	2.88	7.18	0.09	0.13	0.19
C.D. *(P=0.05)*	3.05	6.16	16.03	5.26	8.06	20.31	0.26	0.37	0.55

respectively. This may be due to increased uptake of water associated with reduced loss of water resulting in optimum water balance in the spike. The highest loss-uptake ratio (2.58) was recorded in the spike harvested on 1st August (D_4). In case of floral preservatives, maximum uptake of water (77.42 g) and lowest loss-uptake ratio (1.42) were registered in 500 ppm aluminum sulphate + 4% sucrose (C_6), whereas, the minimum loss of water (72.40 g) was with 50 ppm silver nitrate (C_3). The interaction effect was also found significant and recorded superior at combinations 1st October harvesting + (500 ppm aluminum sulphate + 4 % sucrose) (D_8C_6) for uptake of water and loss-uptake ratio, whereas, D_5C_3 for loss of water. Both aluminum sulphate and sucrose, help in increased uptake and reduced loss of water. These results are in agreement with the findings of Reddy *et al* (1995) and Reddy and Singh (1996) in tuberose.

Fresh weight of spike (g)

Maximum fresh weight (60.72 g) at 14th day was recorded in 15th August (D_5) harvested spikes, which was at par with harvesting dates D_8, D_7, D_3 & D_2 (Table 3). The higher fresh weight might be due to higher water uptake coupled with lowest loss of water. Low temperature and high humidity during October might have reduced transpiration thus lowering water loss from the spikes.

Significantly highest spike fresh weight (68.71 g) was observed with 500 ppm aluminum sulphate + 4% sucrose (C_6), whereas, the lowest fresh weight (44.63 g) was recorded in control (C_{10}). It may be due to the fact that both aluminum sulphate and sucrose improve the water retention of the spike. Sucrose has been shown to act as an oxidisable respiratory substrate and antidesiccant and, thus, increases the fresh weight. Similar results were also obtained by Reddy and Singh (1996) and Bhaskar *et al (*2000) in tuberose.

Percentage of opened and partially opened florets

Maximum percentage of opened florets (46.39 %) was recorded in D_5 (Harvesting at 15th August) and among floral preservatives C_8 (400 ppm 8-HQS+ 4 % sucrose) recorded highest (58.20%). Similarly, for percentage of partial opened florets, maximum (5.34 and 5.11%) was observed in D_6 (Harvesting at 1st September) and C_7 (50 ppm silver nitrate + 4% sucrose), respectively (Table 3). The interaction effect was found non significant for both.

Table 3. Effect of date of harvesting and floral preservatives on fresh weight of spike, percentage of fully opened and partial opened florets during vase life.

Treats	Fresh Weight (g) at 14th days			Opened florets (%) at 12th days			Partial opened florets at 12th days		
	2003	2004	Pooled	2003	2004	Pooled	2003	2004	Pooled
					Date of harvesting				
D_1	50.67	51.17	50.92	26.03	27.63	26.83	3.40(10.59)	3.72(12.84)	3.56(11.69)
D_2	52.40	52.53	52.47	39.37	29.72	34.55	3.70(12.66)	3.81(13.55)	3.75(13.10)
D_3	54.57	50.50	52.53	45.16	42.28	43.72	5.25(26.59)	4.37(18.06)	4.81(22.13)
D_4	44.10	44.10	44.10	37.67	33.07	35.37	3.97(14.75)	3.95(14.59)	3.96(14.67)
D_5	67.60	53.83	60.72	50.24	42.54	46.39	4.35(17.89)	4.59(20.05)	4.47(18.95)
D_6	55.33	45.77	50.55	43.30	28.42	35.86	5.01(24.11)	5.67(31.15	5.34(27.52)
D_7	55.80	51.43	53.62	28.57	33.03	30.80	5.18(25.81)	4.78(21.89)	4.98(23.81)
D_8	59.33	57.33	58.33	31.82	32.29	32.05	4.79(21.98)	3.94(14.51	4.37(18.06)
S.Em.±	0.618	0.607	2.56	0.260	0.305	3.21	0.021	0.025	0.28
C.D. *(P=0.05)*	1.73	1.70	8.55	0.73	0.85	10.71	0.06	0.07	0.93
					Floral preservatives				
C_1	64.96	56.50	60.73	32.62	31.33	31.97	4.32(10.59)	4.49(12.84)	4.40(11.69)
C_2	61.83	59.63	60.73	41.51	36.86	39.19	3.9912.66)	4.06(13.55)	4.03(13.10)
C_3	58.25	47.67	52.96	25.68	16.50	21.09	4.43(26.59)	4.69(18.06)	4.56(22.13)
C_4	47.25	42.71	44.98	51.48	48.89	50.19	4.17(14.75)	3.85(14.59)	4.01(14.67)
C_5	46.63	49.92	48.27	35.42	32.37	33.89	4.76(21.68)	4.17(16.40)	4.47(18.95)
C_6	69.67	67.75	68.71	44.86	42.79	43.83	4.94(23.40)	4.54(19.65)	4.74(21.49)
C_7	50.04	49.38	49.71	27.76	18.81	23.29	5.26(26.68)	4.96(23.63)	5.11(25.13)
C_8	46.79	46.58	46.69	56.53	59.87	58.20	4.05(15.41)	3.95(14.63)	4.00(15.02)
C_9	55.75	47.54	51.65	35.62	29.86	32.74	4.16(16.31)	4.51(19.36)	4.34(17.80)
C_{10}	48.58	40.67	44.63	26.22	18.94	22.58	4.47(19.02)	4.31(17.54)	4.39(18.27)
S.Em.±	0.691	0.679	2.25	0.291	0.341	1.92	0.024	0.028	0.15
C.D. *(P=0.05)*	1.93	1.90	7.20	0.81	0.95	6.15	0.07	0.08	0.49
					Interaction D x C				
S.Em.±	1.95	1.92	4.64	0.82	0.96	5.09	0.07	0.08	0.54
C.D. *(P=0.05)*	5.47	5.37	13.12	2.31	2.70	NS	0.19	0.22	NS

The result may be due to higher uptake of water with low transpiration because of low temperature with slight changes in relative humidity and evapo-transpiration. The 8-HQS has germicidal and chelating properties, which might have reduced the stem blockage and maintained the water conductivity. Sucrose prevents the moisture stress by increasing the osmotic concentration and water absorption. Similar beneficial effect of sucrose was also noted by Mukhopadhyay, (1982); Reddy *et al* (1997); Singh *et al* (1994) and Nagaraju *et al* (2002) in tuberose.

Percentage of neck bent and abscised florets

Significantly lower percentage of neck bent and abscised florets (34.14 & 1.44%) were registered at 15th July (D_3) and 15th June (D_1), respectively, (Table 4). The results might be due to optimum water balance in the spike, which could have lowered the concentration of abscissic acid (ABA) and ethylene. Among floral preservatives, the lowest (24.12 and 2.72%) were observed in C_8 (400 ppm 8-HQS + 4% sucrose) and C_1 (4% sucrose), respectively. The interaction was significant for abscised florets only. The 8-HQS initiates the activities of cytokinin, which might have decreased the ethylene production thereby resulting in lower percent of neck bent florets. Sucrose also antagonizes the effects of abscissic acid in delaying the senescence.

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Table 4 . Effect of date of harvesting and floral preservatives on percentage of neck bent as well as abscised florets at 12th day of vase life of tuberose.

Treatment	Neck bent florets (%)			Abscised florets (%)		
	2003	2004	Pooled	2003	2004	Pooled
			Date of harvesting			
D_1	34.22	36.05	35.14	*1.44(1.07)	1.44(1.06)	1.44(1.07)
D_2	36.06	38.21	37.13	2.62(5.87)	2.60(5.78)	2.61(5.82)
D_3	35.74	32.53	34.14	3.92(14.39)	3.96(14.68)	3.94(14.54)
D_4	39.80	36.59	38.19	4.04(15.32)	3.80(13.44)	3.92(14.37)
D_5	36.82	35.91	36.36	3.79(13.37)	3.93(14.42)	3.86(13.89)
D_6	40.81	39.30	40.05	3.55(11.61)	2.75(6.56)	3.15(8.92)
D_7	37.49	36.26	36.87	4.80(22.06)	4.68(20.90)	4.74(21.48)
D_8	39.55	38.44	38.99	4.35(17.91)	4.29(17.43)	4.32(17.67)
S.Em.±	0.385	0.543	1.00	0.025	0.017	0.15
C.D. *(P=0.05)*	1.08	1.52	3.33	0.07	0.05	0.49
			Floral preservatives			
C_1	33.33	29.61	31.47	2.62(5.85)	2.83(6.99)	2.72(6.40)
C_2	22.99	28.01	25.50	4.57(19.87)	4.37(18.06)	4.47(18.96)
C_3	39.10	53.74	46.42	3.44(10.81)	3.15(8.90)	3.29(9.84)
C_4	35.00	22.61	28.80	4.07(15.53)	3.57(11.77)	3.82(13.59)
C_5	44.90	34.46	39.68	3.85(13.86)	3.15(8.90)	3.50(11.25)
C_6	28.58	31.46	30.02	3.69(12.59)	3.51(11.32)	3.60(11.95)
C_7	53.53	58.73	56.13	2.83(7.03)	3.08(8.46)	2.95(7.73)
C_8	34.88	13.35	24.12	3.65(12.31)	3.30(9.86)	3.47(11.06)
C_9	36.06	36.36	36.21	3.82(13.56)	3.28(9.74)	3.55(11.58)
C_{10}	47.21	58.28	52.75	3.12(8.71)	4.10(15.78)	3.61(12.00)
S.Em.±	0.431	0.607	5.60	0.028	0.020	0.25
C.D. *(P=0.05)*	1.21	1.70	17.91	0.08	0.05	0.79
			Interaction D x C			
S.Em.±	1.22	1.72	7.18	0.08	0.06	0.51
C.D. *(P=0.05)*	3.41	4.81	NS	0.22	0.15	1.44

* A figure out of parentheses indicates square root transformed value

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Short communication

Performance of mosambi sweet orange on different rootstocks grown in laterite soil in West Bengal

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ABSTRACT

A rootstock trial was laid out on sweet orange cultivar 'Mosambi' budded on five rootstocks *viz.*, Jambhiri, Karna Khatta, Kichili, Rangpur lime and Sour orange. Tree growth was maximum on Jambhiri and minimum on Rangpur lime. Fruit yield (both in number and weight) was highest on Karna Khatta, rootstock followed by Rangpur lime while, fruit size and juice content were maximum on Rangpur lime. Total soluble solids and ascorbic acid content were highest in Karna Khatta, while T.S.S. to acid ratio was maximum in Rangpur lime. Foliar nitrogen content was highest in Karna Khatta followed by Rangpur lime. On the basis of four seasons data in respect of yield and fruit quality, Karna Khatta and Rangpur lime were the observed as suitable rootstocks for 'Mosambi' sweet orange grown on laterite soil of West Bengal.

Key words : Mosambi Sweet orange, rootstock, rainfed, laterite soil

In the western part of West Bengal, the soil is red and laterite and climate is somewhat semi-arid, where mosambi sweet orange is performing well under rainfed condition (Ghosh and Chattopadhyay, 1998). To harness beneficial effect of rootstock, the sweet orange was grown on different rootstocks which were standardized suitable for other regions in the country (Kumar Ram and Ganapathy, 1992; Sharma *et al*, 2002; Kusuma Grace *et al*, 2005). For successful cultivation of sweet orange, standardization of suitable rootstock for a locality is of utmost need. Because, a combination which is satisfied under one set of agro-climatic condition, may or may not fail entirely in other condition. Information about suitable rootstock for any sweet orange variety is unavailable for West Bengal, particularly for red lateritic zone, which is emerging as potential area for 'Mosambi' cultivation. Hence, an investigation was undertaken with five rootstocks to find out the suitable rootstock using scion of 'Mosambi' sweet orange.

The trial was laid out (planted) at Regional Research Station, Jhargram (of Bidhan Chandra Krishi Viswavidyalaya) during 1997, in randomized block design with four replications having four plants each. The experimental site was laterite having pH 5.6, available nitrogen 300.0 kg/ha, available phosphorus 30.6 kg/ha and available potassium 101.0 kg/ha. The five rootstocks employed for 'Mosambi' sweet orange were Jambheri, Karna Khatta, Kichili, Rangpur lime and Sour orange maintaining row-to-tow and plant-to-plant distance of 5.0 m apart. Uniform cultural practices were given to all the plants maintained under rainfed condition. The data on growth parameters such as plant height, basal girth of scion and spread of the tree were recorded 7 years after planting. The yield and fruit quality characteristics like fruit weight, juice percentage, T.S.S., acidity and ascorbic acid content were studied for four years (2003 to 2006). The observations on yield and physico-chemical characteristics of fruits were recorded at maturity. The leaves collected in September (Bhargava, 1999) were subjected to analysis of nitrogen following Kjeldahl method (Jackson, 1973), phosphorus by vandomolybdo phosphoric acid method and potassium by flame photometer (Jackson, 1973).

Growth parameters, viz., height and basal girth significantly varied in Mosambi on different rootstocks (Table 1) measured 7 years after planting. Mosambi on Jambhiri rootstock produced vigorous tree, having maximum height and spread, while on Karna Khatta, it was

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Table 1. Effect of rootstocks on plant growth and fruit yield of Mosambi sweet orange

Rootstock	Plant growth 7 year after planting				Number of fruits/plant					Average yield/plant (kg)
	Height (cm)	Basal girth (cm)	Plant spread (cm) East-West	Plant spread (cm) North-South	2003	2004	2005	2006	Average	
Jambhiri	240	20	208	215	3	15	79	83	45	6.6
Karna Khatta	238	19	202	173	75	50	125	113	91	11.7
Kichili	233	20	207	210	13	0	37	51	25	3.2
Rangpur lime	220	20	176	170	22	32	115	83	63	10.3
Sour Orange	226	19	184	179	23	4	39	0	17	2.4
C.D. (*P=0.05*)	4.1	N.S.	3.8	3.9	5.4	3.2	8.8	6.2	5.8	0.7

semi-vigorous and on Rangpur lime, the growth was minimum. Growth of sweet orange tree was maximum on Jambhiri (Jatti Khatti) followed by Karna Khatta while on Rangpur lime rootstock, growth was minimum. Similar observations were made by Mehrotra *et al* (1984) in Punjab.

Fruit production in 'Mosambi' significantly varied on different rootstocks (Table 1). The fruit yield in most of the combinations increased like a tide with one year more followed by less in next year. Maximum number of fruits per tree were produced by 'Mosambi' trees on Karna Khatta irrespective of the years with an average of 91 fruits/tree followed by Rangpur lime (63 fruits/tree). Mosambi on Kichili and sour orange rootstock showed less fruit production. Results from the rootstock trial conducted at various locations, indicated that sweet orange tree on jatti khatti rootstock produced maximum yield (Kumar Ram and Ganapathy, 1992; Sharma *et al,* 2002), while in the present investigation Karna Khatta resulted highest yield constantly. Like number of fruits, fruit yield in 'Mosambi' was highest on Karna Khatta rootstock (11.7 kg/tree) followed by Rangpur lime (10.3 kg/tree) and minimum on sour orange (2.4 kg/tree) and Kichili (3.2 kg/tree).

The fruit weight of Mosambi sweet orange on different rootstocks showed significant differences (Table 2). Fruit weight was maximum on Rangpur lime rootstock (164 g) followed by on jambhiri (146g). Fruit weight was minimum on Kichili and Karna Khatta (127-129 g). For getting premium price, individual fruit weight in orange is considered to be one of the important criteria in West Bengal and other parts of the country. Kusuma Grace *et al* (2005) also recorded highest fruit weight of Sathgudi sweet orange on Rangpur lime rootstock, grown at Tirupati (Andhra Pradesh). Juice content of Mosambi fruit varied significantly on different rootstocks (Table 2). It was highest on Rangpur lime (58%) and lowest on Sour orange and Kharna Khatta (51%). Kusuma Grace *et al* (2005) recorded highest juice volume of Sathgudi fruit on Rangpur lime rootstock.

Total soluble solids (TSS) content of 'Mosambi' sweet orange on different rootstocks varied each other (Table 2). 'Mosambi' on Karna Khatta rootstock showed highest T.S.S. (9.7^{0}B) and lowest on Jambhiri (8.2^{0}B). Acidity content in Mosambi fruit was not differ significantly on different rootstock. T.S.S. : Acid ratio, which determine the organoleptic taste, was more in the fruits from Rangpur lime rootstock followed by Karna Khatta (31.3). However, T.S.S. : acid ratio was not varied so much among the fruits from different rootstocks. Ascorbic acid content in 'Mosambi' fruits greatly differ on different rootstocks. The fruits on Karna Khatta rootstock recorded highest amount of ascorbic acid (64.0 mg/100 ml juice) followed by on Kichili (60.8 mg/100 ml) and lowest on Sour orange (50.3 mg/100 ml).

Foliar phosphorus and potassium content in leaves of 'Mosambi' on different rootstocks were not significantly

Table 2. Effect of rootstocks on physico-chemical characteristics and foliar N, P and K status of Mosambi sweet orange.

Rootstock	Fruit weight (g)	Juice (%)	T.S.S. (^{0}B)	Acidity (%)	T.S.S./ Acid ratio	Ascorbic acid (mg/ 100ml juice)	Nitrogen	Phosphorus	Potassium
Jambhiri	146	53	8.2	0.29	28.3	57.6	2.18	0.21	1.0
Karna Khatta	129	51	9.7	0.31	31.3	64.0	2.94	0.15	0.8
Kichili	127	53	8.3	0.29	28.6	60.8	1.79	0.17	1.2
Rangpur lime	164	58	8.5	0.27	31.5	59.8	2.63	0.20	0.9
Sour Orange	141	51	8.8	0.31	28.4	50.3	1.68	0.15	0.9
C.D. (*P=0.05*)	4.5	1.3	0.2	N.S.	-	1.2	0.40	N.S.	N.S.

differ among themselves (Table 2). However, nitrogen content in leaves was significantly varied among the rootstocks and it was highest in 'Mosambi' on Karna Khatta (2.94%) rootstock which resulted maximum fruits production in every year. Foliar nitrogen content in 'Mosambi' was also higher on Rangpur lime rootstock which gave highest fruit weight with good fruit yield. Differential status of nitrogen in leaves of 'Mosambi' on different rootstocks may be due to differential absorbing ability of the rootstocks. Foliar nitrogen content was lowest in Sour orange (1.68%) followed by Kichili (1.79%), which resulted poor fruit production. It was interestingly noted that there was a direct relationship with the foliar N content and fruit production

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Short communication

Effect of pre-harvest application of GA_3 and PP_{333} as bulb dip and foliar spray on quality and vase life of cut tulip cv. Cassini

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ABSTRACT

An experiment on effect of pre-harvest application of GA_3 and PP_{333} as bulb dip and foliar spray on quality and vase life of cut tulip cv. Cassini was carried out. Healthy scapes of uniform size were cut in a slanting manner at bud colour break stage and placed in conical flasks containing distilled water for vase life studies. Bulb dip in GA_3 (100 ppm) followed by foliar spray of GA_3 (100 ppm) significantly improved overall water uptake, prevented water loss and resulted in maximum water balance. The treatment also exhibited the maximum flower diameter (7.40 cm), scape length (16.26 cm) and vase life (9.33 days). However, the lowest water loss to water uptake ratio was recorded with bulb dip plus foliar spray with 200 ppm GA_3. Data indicated that GA_3 (100 ppm) as bulb dip plus foliar spray proved instrumental in maintaining the quality and vase life of cut tulip as compared to other treatments.

Key words: Cut tulip, quality, vase life, gibberellic acid, paclobutrazol

The tulip (*Tulipa gesneriana* L.) is excellent for cut flowers, garden display and pot culture. In India, tulips thrive well in temperate regions of Jammu & Kashmir, Himachal Pradesh, Uttranchal and other similar hilly regions. There is a good scope for growing tulips for cut flowers in temperate regions. The short vase life of tulip, however, is a major bottleneck in exploiting its utility on a wider scale and even restricts distant marketing. Therefore, post harvest handling plays an important role in enhancing keeping quality of cut flowers. Post harvest application of various growth regulators have been used in vase solutions to enhance the vase life of cut flowers (Salvi *et al*, 1999). However, pre harvest [1]Plant Physiology Section, Division of Post harvest Technology management is also equally important to improve the post harvest behavior and quality Gibbrellic acid (GA_3) and paclobutrazol (PP_{333}) have been reported to increase the yield and post harvest quality of many flowers (Harbaugh and Wilfret, 1979; Singh *et al*, 1999). Paclobutrazol results in retardation of vegetative growth and diversion of assimilates to reproductive growth, giving increased yield potential with better quality flowers. Keeping above facts in view, the present investigation was carried out to analyze the effect of pre harvest application of GA_3 and paclobutrazol on post harvest behavior and vase life of cut tulip cv. Cassini.

The present experiment was carried out at the Division of Floriculture, Medicinal and Aromatic Plants, SKUAST-K, Shalimar, Srinagar during 2003-04. Healthy and uniform sized bulbs of tulip cv. Cassini were dipped in different concentrations of GA_3 (100, 200 and 300 ppm) and PP_{333} (10, 20 and 30 ppm) for 30 minutes. The growing media prepared by mixing soil + compost + sand in the ratio of 2:1:1 was filled in clay pots measuring 20 cm in diameter. Air dried bulbs were planted in pots following the randomized block design. When plants reached 3-leaf stage, three concentrations of GA_3 (100, 200 and 300 ppm) and PP_{333} (10, 20 and 30 ppm) were applied as foliar spray to wet the leaves completely. There were a total of 19 treatments including control (distilled water). Uniform cultural practices like application of fertilizers, weeding, irrigation and plant protection measures were adopted. The healthy looking scapes of uniform size were cut in a slanting manner at bud colour break stage leaving only one leaf on each scape. After taking the initial weight, scapes were placed in conical flasks containing 250 ml of distilled water. All the treatments were replicated thrice with five flasks in

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each replication. The weight of each flask, with and without flower scape, were recorded on alternate days and per cent fresh weight gain, cumulative water uptake, water loss, water balance and water loss-water uptake ratio were calculated (Venkatarayappa *et al*, 1980). Days taken to flower, flower diameter, scape length and vase life calculated from the day of full flower to the day when petals expressed first sign of wilting, were also recorded and the method of Gomez and Gomez (1984) was applied for analysis of variance.

Perusal of the data presented in table 1 revealed that per cent fresh weight gain of scapes decreased due to the bulb dip treatments in GA_3 as well as PP_{333}. However, scapes which received foliar sprays of GA_3 and PP_{333} showed significant increase in fresh weight gain. In case of combined application of bulb dip + foliar spray, only lower doses of GA_3 (100 ppm) and PP_{333} (10 ppm) increased the fresh weight gain while higher doses of GA_3 (200 and 300 ppm) and PP_{333} (20 and 30 ppm) significantly reduced the fresh weight gain. Increased fresh weight gain of tulip scapes by foliar sprays of GA_3 could be attributed to the ability of GA_3 to maintain higher soluble sugar content in the perianth tissue and membrane properties (Sultan and Farooq, 1999). Data also showed that both cumulative water uptake and water loss increased remarkably due to various hormonal treatments, however, 100 ppm of GA_3 applied as bulb dip plus foliar spray exhibited the maximum water balance (14.17 g/scape) with minimum water loss-water uptake ratio (0.72) followed by foliar spray of 100 ppm GA_3. This may be due to the fact that GA_3 increases water uptake capacity and reduces the water loss by maintaining better water loss-water uptake ratio. These results are in agreement with the findings of Rekha *et al* (2001) in gladiolus and Emongor (2004) in lilium.

Pre harvest application of plant growth regulators significantly influenced the cut flower quality and vase life of tulip (Table 2). It is obvious from the data that days taken to flower decreased due to application of GA_3 as well as PP_{333}, GA_3 application also resulted in earliness of flowering when given as foliar spray. Similar results were also reported by Nasr and Shalabi (1996) in *Zantedeschia.* Both GA_3 and PP_{333} treatments also caused an increase in diameter of flowers although these results were insignificant. Pre harvest application of GA_3 resulted in an increased scape length whereas PP_{333} caused a decrease in scape length. The maximum scape length (16.26 cm) was recorded with (GA_3 100 ppm) as bulb dip plus foliar spray followed by GA_3 (300 ppm) as foliar spray. This rapid growth by the application of GA_3 is due to the higher number of cells formed as well as elongation of individual cell by way of more utilization of Photosynthates (Su and Kwack, 1989; Ramesh *et al*, 2001; Sharma *et al*, 2001). Shortened scape length due to the application of PP_{333} is also in accordance with the result of Kwack and Kwack (1990). Results pertaining to the vase life revealed that foliar application of GA_3 significantly increased the vase life of cut tulip while PP_{333} resulted in reduced vase life of tulip. However, the maximum vase life (9.33 day) was recorded with bulb dip plus foliar spray of 100 ppm of GA_3 followed by the foliar spray of 100 ppm GA_3 alone. Similar results were reported by Dutta *et al* (1993) in chrysanthemum, Ichimura and Goto (2000) in narcissus and Gaur *et al* (2003) in gladiolus.

It is concluded from the findings of the present experiment that longer vase life and maximum flower diameter of tulip cut flowers can be achieved by application of 100 ppm GA_3 as bulb dip and /or foliar spray to maintain high water balance through low water loss –water uptake ratio.

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Short communication

Effect of bunch-trimming on yield and quality in banana

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ABSTRACT

The experiment consisted of different intensities of hand removal viz. 1,2 and 3 hands (H_1, H_2 and H_3 respectively) and time of hand removal i.e., immediately after opening of last hand (T_1), one week after opening of last hand (T_2), and two weeks after opening of last hand (T_3). Results were statistically analysed using augmented 2 factor factorial CRD. The time of hand removal did not show any significant difference on yield while hand weight, finger weight, finger length, finger diameter and volume of finger increased with the increase in number of hands removed. It is suggested that removal of three hands between one and two weeks after opening of last hand is beneficial for improving yield and finger quality of banana cv. Martaman (*Musa* AAB).

Key words: Banana (*Musa* AAB) , bunch trimming, production, quality

INTRODUCTION

Basal hands of a banana bunch are often larger in size than the terminal hands. These are usually discarded or sold as third quality fruits in the market. Thus, at least two or three hands in a bunch fail to reach the finger quality standards required for the specialized markets thereby reducing income to the producers. Dehanding consists of removing two or three terminal hands of each bunch and is a routine practice in banana production system for export. By removing the terminal hands, it may be expected that dry matter would be redistributed among the remaining hands of the bunch thus helping to increase the size of the remaining hands (Rodriguez *et al*, 1988). Keeping the above aspects in view the present investigation was carried out.

MATERIAL AND METHODS

The experiment was conducted in the Research Station of All India Coordinated Research Project on Tropical Fruits at Mondouri of Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal on the dessert cultivar, Martaman (*Musa* AAB). One hundred and twenty four (124) plants of cv. Martaman spaced at 1.8 m ´ 1.8 m were selected for bunch trimming with three replications laid out in augmented 2 factor factorial CRD. The experiment consisted of different intensities of hand removal viz. 1, 2, or 3 hands (H_1, H_2 and H_3 respectively) and time of hand removal viz. immediately after opening of hand, one week after opening of last hand, two weeks after opening of last hand (T_1, T_2, T_3 respectively) along with control. Allocation of bunch trimming treatments were done on the bunches which had opened on the same day with uniform length, finger size and having nine hands. The floral remnants and male buds were removed. Observations on yield, hand weight, finger weight, finger volume, finger density, pulp weight, peel weight, pulp/peel ratio, pulp thickness, peel thickness, TSS, sugar and acidity were recorded. For statistical analysis, Principal Component Analysis was followed, based on correlation matrix.

RESULTS AND DISCUSSION

It was evident that hand removal had significant effect on bunch weight, yield, hand weight, finger weight, finger length, diameter, pulp weight, peel weight, pulp thickness, peel thickness, total sugar, reducing and non-reducing sugar, acidity and TSS/acid ratio. The highest bunch weight of 14.95 kg was recorded with removal of one hand (H_1). Time of hand removal and interaction effect of number of hands removed and time of hand removal (H ´ T) significantly affected bunch weight. Bunch weight of 15.14 kg was recorded with removal of one hand after one week of opening of last hand (H_1T_2) followed by removal of one hand after two weeks of opening of last hand (H_1T_3) and immediately after opening of last hand (H1T1).

However, the untrimmed plants yielded a maximum bunch yield of 15.20 kg as compared to trimmed bunches. Among the various intensities of hand removal, one hand removal (H_1) showed yield of 46.14 t/ha. The time of hand removal did not show any significant difference on yield

Table 1. Effect of intensity and time of hand removal on bunch characters

Treatment	Weight of bunch (kg)	Yield (t/ha)	Weight of hand (kg)	Weight of finger (g)
Number of hand removal (H)				
H_1	14.95	46.14	1.821	141.99
H_2	13.17	40.63	1.833	145.33
H_3	12.75	39.36	2.002	153.27
S.Em (±)	0.168	0.518	0.004	0.592
CD *(P=0.05)*	0.496	1.528	0.012	1.746
Time of hand removal (T)				
T_1	13.61	41.99	1.862	144.85
T_2	13.63	42.06	1.885	149.43
T_3	13.64	42.08	1.909	146.30
S.Em (±)	0.168	0.518	0.004	0.592
CD *(P=0.05)*	NS	NS	NS	1.746

Treatment	Length of finger (cm)	Diameter of finger (cm)	Volume of finger (cc)	Density of finger (g/cc)
Number of hand removal (H)				
H_1	11.74	4.02	146.6	0.969
H_2	11.89	4.11	150.60	0.965
H_3	12.20	4.25	158.17	0.968
S.Em (±)	0.038	0.034	0.559	0.001
CD *(P=0.05)*	0.112	0.100	1.649	0.003
Time of hand removal (T)				
T_1	11.86	4.09	150.02	0.965
T_2	11.96	4.18	154.23	0.969
T_3	12.00	4.11	151.13	0.967
S.Em (±)	0.038	0.034	0.559	0.001
CD *(P=0.05)*	0.112	NS	1.649	0.003

Treatment	Total soluble solids (^{0}birx)	Total sugar (%)	Reducing sugar (%)	Non-reducing sugar (%)	Acidity (%)	TSS: Acidity ratio
Number of hand removal (H)						
H_1	18.35	16.29	8.25	7.63	0.482	38.24
H_2	18.36	16.44	8.62	7.43	0.494	37.15
H_3	18.33	16.78	8.83	7.55	0.527	34.75
S.Em (±)	0.041	0.009	0.007	0.01	0.003	0.183
CD *(P=0.05)*	NS	0.027	0.021	0.029	0.009	0.540
Time of hand removal (T)						
T_1	18.35	16.38	8.49	7.49	0.486	37.93
T_2	18.34	16.46	8.55	7.51	0.504	36.41
T_3	18.34	16.67	8.66	7.61	0.513	35.80
S.Em (±)	0.041	0.009	0.007	0.010	0.003	0.183
CD *(P=0.05)*						
Control vs Rest						
S.Em (±)	0.137	0.031	0.025	0.036	0.010	0.645
CD *(P=0.05)*	0.286	0.065	0.052	0.075	0.021	1.345

Note: H_1 = Removal of one hand, H_2 = Removal of two hands and H_3 = Removal of three hands; T_1 =- Removal of hand (s) immediately after opening of last hand, T_2 = Removal of hand (s) one week after opening of last hand, and T_3 = Removal of hand (s) two weeks after opening of last hand

although hand weight, finger weight, finger length, finger diameter and volume of finger increased with the increase in number of hands removed. Increase in fruit weight due to dehanding might be due to higher rate of fruit filling because of reduction in sink size (Jullien *et al*, 2001). Removal of one hand showed highest finger density of 0.969 g/cc. On the contrary, pulp weight, peel weight, pulp thickness, total sugar and reducing sugar improved significantly with the increasing intensity of hand removal. But in case of acidity content and TSS/acid ratio, the data showed a reverse pattern i.e., removal of one hand (H_1) produced fruits having lowest acidity (0.482%) and higher TSS/acid ratio (38.24) compared to two hands (H_2) and three hands (H_3) removal.

Hand removal after two weeks of opening of last hand produced maximum hand weight (1.909 kg), finger weight (149.43 g), finger length (12.0 cm), pulp: peel ratio (3.06) and also the sugar content of fruit. Finger diameter (4.18 cm), finger volume (154.23 cc), density of finger (0.969 g/cc), pulp weight (112.169), peel weight (37.27 g) and pulp thickness (3.91 cm) were higher in T_2

Table 2. Effect of intensity and time of hand removal and their interaction on finger parameters

Treatment	Weight of pulp (g)	Weight of peel (g)	Pulp : Peel ratio	Pulp thickness (cm)	Peel thickness (cm)
Number of hand removal (H)					
H_1	105.88	36.11	2.94	3.73	0.271
H_2	109.05	36.28	3.01	3.83	0.273
H_3	115.87	37.15	3.12	3.98	0.269
S.Em (±)	0.410	0.709	0.066	0.034	0.004
CD *(P=0.05)*	1.209	NS	NS	0.100	NS
Time of hand removal (T)					
T_1	108.59	36.26	3.00	3.81	0.275
T_2	112.16	37.27	3.01	3.91	0.270
T_3	110.05	36.00	3.06	3.82	0.269
S.Em (±)	0.410	0.709	0.066	0.034	0.004
CD *(P=0.05)*	1.209	NS	NS	NS	NS
Control vs Rest					
S.Em (±)	1.427	2.393	0.222	0.120	0.014
CD *(P=0.05)*	2.977	4.992	NS	0.250	0.029

Note:H_1= Removal of one hand, H_2 = Removal of two hands and H_3 = Removal of three hands; T_1 =- Removal of hand (s) immediately after opening of last hand, T_2 = Removal of hand (s) one week after opening of last hand, and T_3 = Removal of hand (s) two weeks after opening of last hand

treatment. Time of hand removal did not show any significant variation in TSS content.

Interaction effect of number of hand removal and time of hand removal significantly affected bunch weight, hand weight, finger weight, finger length, finger diameter, finger volume, density of finger, pulp weight, peel weight, pulp: peel ratio, pulp thickness, TSS, total sugar, reducing sugar and TSS/acid ratio. In respect of bunch weight and yield the untrimmed bunches yielded maximum. This result is supported by the findings of Irizarry *et al* (1992) who reported that three hands removal reduced total yield. Mandal and Sharma (2000) also reported that removal of 1, 2 and 3 lower hands reduced yield by 9, 12.7 and 17.4%, respectively in cultivar Alpan. Removal of three hands after two weeks of opening of last hand (H_3T_3) produced fruits with maximum hand weight (2.020 kg) followed by H_3T_1. Removal of the hands after one week of opening of last hand (H_3T_2) recorded maximum finger weight (156.33) followed by H_3T_3 and H_3T_1 treatments. Control plants yielded the lowest finger weight (119.09 gm) as compared to treatment of hand removal irrespective of its time of removal. H_3T_3 also produced fruits with maximum length (12.33 cm), finger diameter (4.28 cm), pulp: peel ratio (3.15), and pulp thickness (4.01 cm). Arcila *et al* (2002) found that longer size fruit was attained with hand tear off at 20 days after flowering and leaving 4-6 hands per bunch in banana hybrid FHIA-21. Removal of three hands after one week of opening of last hand (H_3T_2) produced fruits with maximum volume (160.93 cc) closely followed by H_3T_3. The same interaction (H_3T_2) proved beneficial in respect of density of finger and also pulp weight. Total sugar content was highest (16.85) in H_3T_2 interaction. In respect of TSS : acid ratio, H_1T_1 i.e., removal of one hand immediately after opening of last hand proved to be the best. Loss of biomass was partially compensated by increasing fruit weight, length and circumference. Treatments of hand removal at different time increased fruit weight, length and diameter through redistribution of dry matter content by reducing competition for photosynthate among the different hands.

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Short communication

Effect of various nursery media on onion seedlings development

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ABSTRACT

A field experiment was conducted to standardize the nursery raising technique for onion at the Horticulture Research Farm, Department of Horticulture, Allahabad Agricultural Institute - Deemed University, Allahabad, during 2005-2006. The treatments comprised combinations of soil, sand, FYM and vermicompost. Altogether, 14 treatments were applied in a randomized block design with three replications. Hundred percent germination was found with a combination of soil, sand and FYM in proportions of 2:1:2 & 2:2:1, and, 1:1:1 & 2:2:1 Soil:Sand:Vermicompost. Among all the treatments, the combination of soil 2 parts, sand 1 part and FYM 2 parts, significantly influenced growth and health of seedlings and produced the maximum seedling height (11.42 cm), stem diameter (0.33 cm), root length (10.86 cm), shoot fresh weight (6.96 g), root fresh weight (3.22 g), total seedling fresh weight (10.18 g), shoot dry weight (3.95 g), root dry weight (1.53 g) and total seedling dry weight (5.48g). Highest benefit:cost ratio of 3.72 was also seen in this treatment combination.

Key words: Onion, vermicompost, FYM, nursery, seedlings

Onion (*Allium cepa* L.), an important member of the genus *Allium* of the family Alliaceae, is believed to have originated in Uzbekistan. India ranks second in the world in area and production after China, and, third in export after the Netherlands and Spain. It is an important vegetable crop of our country under an area of 4.81 lakh hectares, producing 54.61 lakh tonnes of bulbs both for local consumption and export. India exported 3,33,349 tonnes valued at Rs.20,216 lakh (Singh, 2005). Onion bulbs are rich in phosphorus, calcium, carbohydrates and Vitamin C.

Nursery is a place where seedlings are grown to be transplanted in the field. In India, the traditional method of nursery management under open-field condition is completely dependent on vagaries of nature and about 15-20 % seedlings are damaged. Therefore, it is necessary to standardize the nursery raising technique in a scientific way to obtain healthy and vigorous seedling for the growers. In raising a vegetable nursery, rooting and growth media are the most important factors for growth and development of seedlings and the root. But, under mostly open-field conditions, farmers use only soil and FYM in an inadequate proportion. In place of FYM several other organic manures like vermicompost, poultry manure, NADEP compost etc., are available which could be utilized for production of better and healthy seedlings. These manures are easily available, retain sufficient water and air and allow sufficient drainage, thus, providing a congenial rhizosphere for better root-growth. Moreover, these nursery media improve water holding capacity of the soil under open-field conditions. With this in view sand, soil, FYM and vermicompost were used in this investigation in various proportions to accomplish better growth and seedling production in onion.

The experiment was conducted at the Vegetable Research Farm, Department of Horticulture, Allahabad Agricultural Institute-Deemed University, Allahabad (U.P.), during the rabi season of 2005. Onion variety Pusa Red was used in the experiment. Fourteen treatments comprising soil, sand, FYM (Farm Yard Manure) and VC (Vermicompost) were replicated three times. The treatment combinations were T_1 : Soil + sand + FYM (1:1:1), T_2 : Soil + sand + FYM (1:1:2), T_3 : Soil + sand + FYM (1:2:1), T_4 : Soil + sand + FYM (1:2:2), T_5 : Soil + sand + FYM (2:1:1), T_6 : Soil + sand + FYM (2:1:2), T_7 : Soil + sand + FYM (2:2:1),T_8 : Soil + sand + VC (1:1:1), T_9 : Soil + sand + VC (1:1:2),T_{10} : Soil + sand + VC (1:2:1), T_{11} : Soil + sand + VC (1:2:2), T_{12} : Soil + sand + VC (2:2:1), T_{13} : Soil + sand + VC (2:1:2) and T_{14} : Soil + sand + VC (2:2:1). The treatments were laid out in a randomized block design with a nursery plot size 1m x 1 m. Observations were recorded on ten randomly selected plants from each plot for various characters, viz., percent germination at 8, 9, 10 and 11 days after sowing (DAS), seedling height (at 15, 35 and 45 DAS), stem diameter, seedling fresh and dry weight, root and shoot fresh and dry weights at 45 DAS.

Table 1. Influence of various nursery media on raising onion seedlings

Treatment	% germination at 10 DAS	Seedling height (cm)	Stem diameter (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Total seedlings fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Total seedlings dry weight (g)
T_1	70.00	7.09	0.15	7.03	2.86	1.85	4.71	0.49	0.71	1.65
T_2	84.67	8.18	0.18	7.83	3.00	1.89	4.89	1.12	0.83	1.95
T_3	84.33	7.91	0.18	7.61	3.00	1.88	4.88	1.06	0.72	1.78
T_4	87.67	8.50	0.20	8.13	3.27	2.16	5.44	1.30	0.93	2.23
T_5	86.00	8.26	0.19	8.03	3.11	2.05	5.16	1.20	0.84	2.04
T_6	100.00	11.42	0.33	10.86	6.96	3.22	10.18	3.95	1.53	5.48
T_7	100.00	11.22	0.31	10.70	6.55	3.05	9.60	3.57	1.50	5.07
T_8	100.00	10.32	0.26	10.39	4.74	2.77	7.51	2.39	1.44	3.83
T_9	99.33	9.35	0.23	10.02	4.44	2.61	7.05	1.97	1.24	3.21
T_{10}	96.00	8.62	0.21	9.81	3.72	2.28	5.99	1.51	1.04	2.55
T_{11}	99.00	9.14	0.22	9.87	3.89	2.38	6.27	1.52	1.22	2.73
T_{12}	99.00	9.16	0.23	9.88	4.27	2.44	6.71	1.90	1.22	3.12
T_{13}	92.67	8.55	0.21	9.36	3.61	2.22	5.82	1.45	0.99	2.44
T_{14}	100.00	9.56	0.24	10.25	4.44	2.66	7.10	2.16	1.35	3.52
F-Test	S	S	S	S	S	S	S	S	S	S
SEd ±	1.95	0.22	0.02	0.15	0.09	0.06	0.09	0.06	0.03	0.07
CD (*P=0.05*)	4.01	0.45	0.03	0.32	0.18	0.12	0.19	0.12	0.07	0.15

Note: Parameters were recorded at 45 days after sowing except germination percentage

All the treatments showed significant differences for traits like germination percentage, seedling height, seedling fresh and dry weight , stem diameter, root length, fresh and dry weights of roots and shoots (Table 1).

Among the various nursery media, the best performance obtained with application of soil 2 part + sand 1 part + FYM 2 part was found to be significantly superior to the other treatments. This could be due to availability of sufficient nutrient content in FYM. FYM, in ideal combination with soil and sand, created healthy rhizosphere adequate in physico-chemical and biological properties. This combination may have resulted in better growth and seedling production in onion. Similar findings were also reported by Booij *et al* (1985), Ponwell *et al* (1991), Baruah (1997), Boff *et al* (2005) and Tathan (1997). The highest net return of Rs.36560 / 500 m^2 and cost: benefit of 1:3:72 was obtained with application of 2 parts soil + 1 part sand + 2 parts FYM, followed by 2 part soil + 2 part sand + 1 part FYM with a net return of Rs. 36460 / 500 m^2 and cost: benefit ratio of 1:3.71(Table 2). This is also in agreement with the work of Awghad *et al* (1994) in onion.

Table 2. Economics of various treatments imposed

Treatment	Cost of cultivation of raising seedlings for 1 ha(Rs)	Gross returns (Rs.)	Net profit (Rs/ha)	Cost: Benefit ratio
T_1	12600	35000	22400	1:2.77
T_2	13160	42250	29090	1:3.21
T_3	13160	42100	28940	1:3.19
T_4	13720	43800	30080	1:3.19
T_5	12880	43000	30120	1:3.30
T_6	13440	50000	36560	1:3.72
T_7	13440	49900	36460	1:3.71
T_8	14280	49850	35570	1:3.49
T_9	16520	49500	32980	1:2.99
T_{10}	14840	48000	33160	1:3.23
T_{11}	17080	49500	32420	1:2.89
T_{12}	14280	49450	35170	1:3.46
T_{13}	16800	46000	29200	1:2.73
T_{14}	15120	49800	34680	1:3.29

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Event highlights

BRAINSTORMING SESSION ON PROBLEMS AND PROSPECTS OF MARKETING HORTICULTURAL CROPS

JULY 12-13, 2007

A two-day brainstorming session was organized jointly by the Society for Promotion of Horticulture (SPH) and the Indian Institute of Horticulture Research (IIHR), Hessaraghatta, Bangalore, on the 12th and 13th July 2007 at the IIHR auditorium to address issues related to marketing of horticultural crops. Dr. M. R. Hegde, Co-coordinator, Brainstorming Session, welcomed the participants/delegates and introduced the topic during the inaugural session. Dr. P. G. Chengappa, Vice-chancellor, University of Agricultural Sciences (UAS), Bangalore, inaugurated the session and in his address, highlighted the changing scenario in marketing horticultural crops in the light of emergence of new players, like retailers, and the challenges that lay ahead. He stressed the need for streamlining the market chain and for the retail chain to help the farming community. Dr. S. Bisaliah, former Vice-chancellor, UAS, Bangalore, and Sri. B.S. Ramaprasad, Executive Director, Jala Swamvardhana Yojana Sangha, Bangalore, also made their remarks during the inaugural session. Both the speakers emphasized the need for a remunerative market-price and outlined policy changes needed for better market-network. Dr S. D. Shikhamany, President, SPH and Director, IIHR, Bangalore, in his presidential address remarked that production technologies were crucial for supply of quality-produce and emphasized the onus on farmers to supply appropriately for meeting the demand of different markets. He opined that the chief problem of marketing and stressed the need to address it, including exports. Dr. G. S. Prakash, General Secretary, SPH, Bangalore, proposed a formal vote of thanks. Over 200 delegates from various governmental and non-governmental organizations participated in the brainstorming meeting**.** Important **recommendations** that emerged from the meeting are as follows.

1. Need for regulation of production through acreage-control in order to reduce frequency of occurrence of glut in the market. Further, farmers should re-orient themselves to a demand-based production, i.e., to work out the requirement of the market and meet it rather than producing without plan and then trying to get a market for the produce.
2. Quality parameter for both domestic and international market needs to be met in fruits, flowers and vegetables through adoption improved production practices.
3. It is suggested to carry out systematic Pest Risk Analysis (PRA) on major export- oriented crops and develop Indian standards on par with the existing CODEX and European standards to help the exporters.
4. It was recommended to replicate the SAFAL model, which works with the objective of providing backward linkage, auction market and forward linkage.
5. Reducing the length of market-chain is an essential component for increasing farmers' share in the consumer rupee. This can happen even by individual effort.
6. Problems related to marketing need to be addressed by use of concepts like 'Contact' and 'Contract' farming in the supply chain management of fruits and vegetables.
7. There is a need to have better backward linkage so that both the farmers and the consumers benefit. Backward linkages with trained - women Self Help Groups (SHG) would be helpful in getting fresh and quality produce of fruits and vegetables.
8. As regards medicinal crops sector, risk of domestic price fluctuation due to restrictive trade practices, lack of information flow, crop failure, malpractices by middlemen, unqualified consultants in finance, quality of the planting material and testing of active ingredients, lack of processing facilities and limited buyers and sellers, were expressed as problems hindering growth of this sector.
9. It was also recommended to aim for a multi-stakeholder platform for the medicinal plant sector, encouragement

of cooperatives or producers' companies, establishment of common quality-standards, soft-credit and creation of herbal parks and storage facilities. These flaws in the present contract farming need to be addressed.

10. The successful model adopted by FRLHT by bringing together farmers in the form of groups and women SHG was recommended for replication for improving livelihood options of the medicinal crop collectors and cultivators.

11. Value-addition can be multidimensional. It is customer-centric and integration along the supply chain is the most important aspect of value-addition. Making available the produce in good packing during off-season and at the right place is also value-addition. It is important to have a small model unit as a focal point while sourcing raw material locally.

12. Preference of the international market in terms of size, colour and shape of flowers and packing dictated by different countries needs to be borne in mind when while exporting the produce.

13. In the case of export of mango, high air-freight charge is the main constraint. In order to prolong the shelf-life of mango, it was recommended to evolve a protocol for prolonging storage-life of mango to enable sea shipment to various countries, especially, Europe.

14. In the case of gherkin, the main constraints in export are competition from China and Vietnam, higher tariff, fruit-fly and borer problems, and, withdrawal of LC terms for payment. Gherkin, being a condiment, does not command a premium price. 'Made in India' label in bottling is not yet accepted in EU and USA. It was recommended to arrange for VAT refund and, also, to extend AEZ benefits to exporters.

15. Regarding export-problems in Anthurium, it was felt that inadequate supply is the only constraint. There is no incentive for export as the price realized in domestic market is much higher than that in the international market.

16. Constraints like higher rate of interest compared to other countries, unorganized marketing, inconsistent export policies and the problem of market-cess are hindering the growth of export of horticultural crops. Government intervention for identifying markets with better bilateral understanding is required.

17. In Agri-Export Zones (AEZ), the government has not made clear the various mandate crops to be included.

18. Role of export-promotion institutions like the Agriculture and Processed Food Products Export Development Authority (APEDA) and Karnataka State Agricultural Produce Processing and Export, Corporation Ltd (KAPPEC), Bangalore was highlighted and the need for further strengthening the activities of these institutions was stressed.

19. SAUs and ICAR institutions need to create a good marketing information system to study implications of different policies and their impact on volatile agricultural-commodity prices by adopting a holistic approach. They need to stress aspects like produce-for-the-market (acceptance), comparative advantage, diversification of markets, providing market information, follow-up of recommendations made in workshops / meetings at the State and Central level. It was recommended to have better price/demand forecasting models in various commodities to address marketing problems efficiently.

20. In Mango, research efforts are needed to address problems like skin-browning in cv. Banganapalli and spongy-tissue in varieties procured from the Ratnagiri region of Maharashtra.

21. It was recommended to make use of promotional policies of APEDA, like, financial assistance schemes on market-development, infrastructure- development, quality, research and development.

22. Assistance under AEZ, efforts made by APEDA in promotion of 'produce of India' logo, national-level program on systematizing organic production of horticultural crops, launching organic standards and accreditation policy, 'Indian Organic' logo, e-commerce and Food Safety program, etc. need to be intensified.

23. It was recommended to make use of schemes under operation in various banks like State Bank of India (SBI) and Canara Bank for promotion of marketing horticultural crops.

FORTHCOMING EVENTS

Event	Contact details
II International Conference on Vegetable Crops - ICV2008 April 14-18, 2008, Fortaleza (Brazil)	Dr. Fernando Antoni Souza de Aragão EMBRAPA CNPAT, Rua Dra. Sara Mesquita 2270 Planalto Pici, Fortaleza CE, Brazil Phone: (55)32991972, Fax: (55)32991803 E-mail: aragao@cnpat.embrapa.br
XII International Symposium on Virus Diseases in Ornamentals April 20-24, 2008, Haarlem (Netherlands)	Dr. Ellis Meekes, Sotaweg 25 PO Box 40, 2371 AA Roelofarendsveen Netherlands Phone: (31)71-3326236 E-mail symposium: isvdop12@wur.nl Web: http://www.plantenvirologie.nl/ISVDOP12/
XI International Symposium on the Processing Tomato June 8-11, 2008, Toronto (Canada)	Dr. Jane Graham, Ontario Food Processors Association c/o Janisse Routledge, 7660 Mill Rd. Guelph, ONT N1H 6J1, Canada Phone: (1)5197675594, Fax: (1)5197634164 E-mail symposium: 2008worldcongress@opvg.org Web: http://www.worldtomatocongress.com/
IX International Symposium on Integrating Canopy, Rootstock and Environmental Physiology in Orchard Systems August 4-8, 2008, Geneva, NY (United States of America)	Dr. Terence L. Robinson, Dept. Horticultural Science 630 W. North Street, Geneva, NY 14456 United States of America Phone: (1)315-787-2227, Fax: (1)315-787-2216 E-mail: tlr1@cornell.edu
IX International Symposium on Postharvest of Ornamentals August 11-14, 2008, Aarhus (Denmark)	Dr. Carl-Otto Ottosen, Department of Horticulture Aarhus University, Kirstinebjergvej 10 5792 Aarslev, Denmark Phone: (45)89993313 E-mail: co.ottosen@agrsci.dk Web: http://www.postharvestsymposium.dk
VI International Symposium on In Vitro Culture and Horticultural Breeding August 24-28, 2008, Brisbane (Australia)	Prof. Acram Taji, Agronomy & Soil Science Group University of New England Armidale, NSW 2351, Australia Phone: (61)267732869, Fax: (61)267733238 E-mail: ataji@metz.une.edu.au Web: http://www.une.edu.au/campus/confco/ivchb2008/
I International Symposium on Biotechnology of Fruit Species September 1-5, 2008, Dresden, Pillnitz (Germany)	Dr. Viola Hanke, Baz, Institute for Fruit Breeding Pillnitzer Platz 3a, 01326 Dresden, Germany Phone: (49)3512.616.214, Fax: (49)3512.616.213 E-mail: v.hanke@bafz.de Web: http://www.biotechfruit2008.bafz.de
IV Balkan Symposium on Vegetables and Potatoes September 9-12, 2008, Sadovo (Bulgaria)	Prof. Dr. Liliya Krasteva, Institute of Plant Genetic Re sources 2 Drujba Str., 4122 Sadovo, Bulgaria Phone: (359)32629026, Fax: (359)32629026 E-mail: krasteva.liliya@gmail.com Web: http://www.4bsvp.org/

International Symposium on Tomato in the Tropics September 9-12, 2008, Santa Marta (Colombia)	Prof. Dr. Gerhard Fischer, Universidad Nacional Colombia Facultad de Agronomia, Apartado Aéreo 14490 Bogota, Colombia Phone: (57)13165498 or 3165000ext19041, Fax: (57)13165498 E-mail: gerfischer@gmail.com Fax: (57)24450073 Web: http://www.soccolhort.com/tomato/
International Symposium: Greenhouse Environmental Control and Crop Production in Semi-Arid Regions October 20-24, 2008, Tucson, AZ (United States of America)	Prof. Dr. Gene A. Giacomelli, University of Arizona Controlled Env. Agric. Ctr., 504 Shantz Bldg. Tucson, AZ 85721-0038, United States of America Phone: (1)5206269566, Fax: (1)5206261700, E-mail: giacomel@ag.arizona.edu
IV International Symposium on Tropical and Subtropical Fruits November 3-7, 2008, Bogor (Indonesia)	Dr. Roedhy Poerwanto, Jl. Abiyasa Raya No. 1, Bantarjati, 16143 Bogor, Indonesia Phone: (62)251328942, Fax: (62)251326881 E-mail: roedhy@indo.net.id
II International Symposium on Guava and other Myrtaceae November 10-13, 2008, Mérida (Mexico)	Dr. Wolfgang Rohde, MPIZ, Calf-von-Linné-Weg 10 50829 Koeln, Germany Phone: (49)2215062101, Fax: (49)2215062113 E-mail: rohde@mpiz-koeln.mpg.de
XVI International Symposium on Horticultural Economics and Management December 7-11, 2008, Chiang Mai (Thailand)	Peter J. Batt, Horticulture, Curtin University of Technology GPO box U1987, Perth, WA 6845, Australia Phone: (61)8 9266 7596, Fax: (61)8 9266 3063 E-mail: p.batt@curtin.edu.au Web: http://www.muresk.curtin.edu.au/conference/ishsem
V International Symposium on Horticultural Research, Training and Extension December 7-11, 2008, Chiang Mai (Thailand)	Peter J. Batt, Horticulture, Curtin University of Technology GPO box U1987, Perth, WA 6845, Australia Phone: (61)8 9266 7596, Fax: (61)8 9266 3063 E-mail: p.batt@curtin.edu.au Web: http://www.muresk.curtin.edu.au/conference/ishset
IV International Symposium on Acclimatization and Establishment of Micropropagated Plants. December 8-12, 2008, Bangalore (India)	Dr. Jitendra Prakash, In Vitro International Pvt. Ltd. #12/44, Rajiv Gandhi Nagar Bommanahalli, Bangalore 560 068, India Phone: (91)80 41109273, Fax: (91)80 25727030 E-mail: invitro@bgl.vsnl.net.in Web: http://www.int-tissueculturecconf.org/
II International Symposium on Papaya December 9-12, 2008, Madurai, Tamil Nadu (India)	Dr. N. Kumar, Department of Fruit Crops Horticultural College & Research Institute Priyakulam, 625 604, India Phone: (91)4546231726, Fax: (91)4546231726, E-mail: kumarhort@yahoo.com Web: http://www.ishs-papaya2008.com/

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