Review of Literature

Application of isozyme markers in genetic diversity studies:

Genetic relationship studies are better option of relating the biochemical characteristic among species, variations detected by molecular makers offers a number of advantages over morphological and biochemical as they are generally affected by environment and less representation of loci respectively. However, use of isozymes for cultivar identification is well documented and widely used for genetic relationship study. Ramirez et al. (1985) studied three isozyme systems (cathodal peroxidase, alkaline phosphates and alpha-amylase to characterize and classify six rye (Secale cereale L.) seed samples, study indicated that different electrophoretic patterns of isozymes allowed to distinguish each sample.

In contrast to leaf proteins, seed protein composition is generally not influenced by environment factors (Gardiner et al., 1986) are used as valuable tool for cultivars identification. Wouters and Booy (2000) reported the identification of 68 cultivars of perennial ryegrass (Lolium perenne L.) by using and combining the results of esterase isozyme and total seed protein, the discrimination was based on quantitative differences (relative band intensity rather than qualitative differences), it was further reported that esterase pattern from different seeds of rye grass was very stable.

Fernandez et al. (2005) distinguished 29 cultivars of lily (Lilium spp.) by using eight isozyme systems. Some isozyme banding patterns (IBP) were identified as section specific biochemical markers. The cluster analysis indicated that the lily cultivars could be separated from other Lilium species, except for two L. formonlogi cultivars: ‘Hakuba’ and ‘Hakuko’ which could not be distinguished from each other by the isozyme banding patterns, hence isozyme can provide useful biochemical marker for cultivar identification and to estimate phylogenetic relationship among those lily cultivars.
Messina et al. (1991) reported the utility of isozyme as genetic markers in cultivars identification of Kiwifruit (*Actinidia deliciosa*). Fifty four entries putatively belonging to seven female and two male kiwifruit cultivars were examined for 13 isozyme system (AAT, ACO, GDH, G6PDH, 1DH, MDH, ME, MNR, NDH, 6PGD, PG1, PGM and SKDH). Four isozymes showed identical banding patterns (ACO, MDH, NDH and SKDH) and remaining enzyme systems showed best discriminating power. All the New Zealand cultivars were uniquely identified by simultaneous comparison of the AAT, PGI and PGM zymograms.

The isozyme marker system could be useful in genetic improvement and breeding programme involving inter specific crosses; Carrera and Poverene (1995) discriminated *Helianthus petiotarils* (A wild species) from *Helianthus annuus* by using 7 isozymes (ADH, ACP, EST, GDH, LAP, PGI and PGD). The pattern obtained were compared with zymograms of inbred lines, hybrids and open pollinated varieties of *H. annuus*. The same alleles for EST and SKDH isozymes were found in 60 species, while ACP showed an allele that has not been found is sunflower, the rest of the isozyme system showed both common alleles and characteristic ones for each species. ACP, GDH and PGD were monomorphic in *H. petiotarils*, while ADH and LAP were monomorphic in *H. annuus*. Verron et al. (1993) estimated the genetic distance and genetic diversity among five improved varieties and one wild accession of lily of the valley (*Convallaria majalis* L.) by using isozyme marker systems. Five isozymes namely esterase, acid phosphatase, peroxidase, phosphoglcomutase and superoxide dismutase. Esterase system gave lowest polymorphisms, peroxidase and phosphoglcomutase systems gave highest polymorphism. The study indicated that certain genetic diversity exists among the types of lily of the valley and the isozyme variations may be related to genetic variation.

Isozyme system has been also reported as very attractive marker system for breeding purposes. Avila et al. (2003) studied eighteen isozyme systems for evaluation and characterization of 147 accessions of *Vicia faba* from different
origins, including four known botanical types. The most polymorphic isozyme systems were AAT, FK, PGD, PRX and SOD, which provided a discriminatory tool for evaluating and characterizing collections. The study also detected possible contamination of or some degree of heterozygosity within certain inbred lines. The study discussed the use of allozymic variation in germplasm identification and characterization, breeding programs, and other genetic studies on the species (*Vicia faba*). Although molecular markers are now predominantly used for genetic relationship and trait association studies, isozyme analysis continues to be used as a relative simple and inexpensive method for obtaining genetic information for screening of large germplasm collection.

**Application of DNA based molecular markers system in genetic diversity studies:**

**RAPD marker system:**

Two decades ago, Bostein et al. (1980) describe the first DNA profiling technique, RFLP and RAPD. The major areas of potential marker utilization were defined as:

1. Varietals and percentage identification,
2. Identification of genetic loci affecting quantitative economic traits;
3. Genetic improvement programme, including screening and evaluation of germplasm source resource, introgression, improvement of commercial hybrids and within population selection (Sollar and Beckmann, 1983). The advent of PCR (Saiki et al., 1988) and the resulting exponential increase of marker systems suitable for genetic analysis have given a substantial impetus both for the proliferation of genetic diversity studies, and the initiation of marker assisted selection.

The choice of marker system (i) for genetic diversity studies is driven by several considerations. These include the availability of markers, marginal assay cost, size of experiment and preference between a high average expected heterozygosity and a high effective multiplex ratio (Powell et al., 1996).
DNA markers like restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) have been used in genetic and breeding studies in many plant species (Williams et al., 1993). Random amplified polymorphic DNA (RAPD) markers (Williams et al., 1990) have been used in cultivar analysis, species identification in most plants due to technical simplicity and speed of RAPD methodology. Gepts (1993) compared to restriction fragment length polymorphism (RFLP) markers with RAPD, and elucidated that RAPD can generate markers more rapidly but some loss of information may occur because RAPD markers are usually dominant rather than co-dominant as RFLP markers. Our earlier studies in Dichanthium indicated a high level of variations with RAPD markers (Chandra et al., 2004).

Ever since molecular makers become available in appreciable numbers, many studies have been directed at Hordeum spontaneum, the immediate wild ancestor of cultivated barley. Zhang et al. (1993) typed 268 accessions using isozyme and RFLP marker. While both RFLP and isozymes were highly polymorphic both within and among population, neither the number of alleles per locus nor the average level of diversity differed with either technique. However, the relative amount of within Vs between population components varied greatly between the two marker systems. Isozymes revealed a large amount of within population diversity, whereas RFLP resolved a higher proportion of between population and detected more heterozygosity. More recently Pakniyat et al. (1997) explored the potential of AFLP markers to estimate genetic diversity, as well as to genetically analyze various complex traits. Thirty nine genotypes of H. spontaneum belonging to three geographically separated areas of the fertile crescents discriminated on the basis of AFLP patterns. Owuor et al. (1999) studied on H. spontaneum, used RAPDs to demonstrate strong association between specific loci and soil types, between gene diversity and soil type, and in the frequency with which rare alleles were observed in one soil type over another; Molecular marker have not only proved efficient in the analysis of genetic diversity in space and across eco-geographic gradients but have also been
successfully used to test that common assertion that scientific plant breeding has led to a narrowing is crop diversity overtime (Reeves et al., 1999; Donini et al., 2000).

Punitha and Ranveendran (2004) assessed the genetic diversity in colored and selected white linned cottons using RAPD molecular marker system. A collection of 11 colored cotton (Gossypium hirsutum) genotypes and four white linned genotypes of different origin were evaluated using 32 different RAPD primers, high level of polymorphism (76.31) was detected the cluster analysis showed clear cut separation of colored and white linned genotypes and thus formed three clusters.

Nair et al. (2002) studied the genetic relationship and diversity in 28 prominent Indian sugarcane varieties cultivated under wide range of agro climatic conditions using 25 RAPD markers. The varieties belonging to the one parentage were grouped under different cluster white varieties from different percentages were grouped under the same cluster. The tropical and subtropical identifies of verities also did not contributed to the clustering pattern as individual clusters included varieties from both tropics and subtropics. This showed that genetically similar varieties are present in both regions.

Singh et al. (2006) used RAPD marker system for assessing genetic diversity and species relationship among 28 accessions of egg plant representing five species, these accessions were collected from different part of country. In total 144 polymorphic bands were obtained from 14 random primers, the value of Jaccard’s coefficient ranged from 0.05 to 0.82 dendrogram (by UPGMA) showed that S. incanum is closest to S. melongena followed by S. nigrum and genetically distinct genotypes were identified.

Mantzavinou et al. (2005) estimated genetic diversity of 19 Greek landraces and 9 cultivars of durum wheat [Triticum turgidum L.var. durum
(Desf.)] along with two commercial bread wheat cultivars and one genotype of *Triticum monococcum* L. by using RAPD method. In total 87 seven random primers were screened of that 15 were used for complete analysis. Out of 150 bands obtained 125 bands were polymorphic (82.3%). The cluster analysis revealed that all the genotypes were grouped in one broad cluster where as the *Triticum monococcum* L. cultivar stood apart from all other genotypes.

Molecular markers have clearly played an important role in diversity analysis (Karp *et al.*, 1997). Challenges for the future include the practical exploitation of such markers to relevant ecological and biological questions and for the establishment of core collection in germplasm banks (Gepts, 1995). Opportunities also exists to shift emphasis away from anonymous marker to genes of known function and to focus on factors determining adoptive variation. The narrow genetic base of crop plants is well documented. Tanksley and McCouch (1997) have highlighted the prospects for "unlocking genetic potential from the wild". This strategy is built around the principle that phenotype is not always a good indicator of genotype and that exploitation of genetic resource might be better based on the use of molecular markers. The proposal is that exotic material should be selected by identifying genotypes with the greatest number of unique alleles displayed by DNA profiling and the approach is of particular significance for character that show quantitative inheritance, since the genetic control of these can be complex. The application of molecular and genetic mapping will facilitate access to a broader spectrum of genetic variation and should play a significant role in promoting the sustainable use of genetic resources. Perhaps more importantly exploitation of naturally occurring variation will have an important role in unraveling gene functions (functional genomics). The major advantages of DNA marker over conventional marker system in that, they are practically unlimited in numbers, they are neither growth stage specific nor they are subjected to either environment interactions or to epistatic or pleiotropic effects.
ISSR molecular marker system:

Zietkiewicz et al. (1994) reported this technique for the first time, where microsatellites anchored at the 3' end are used for amplifying genomic DNA. They are mostly dominant markers. Numbers of primers can be synthesized for various combinations of di-, tri-, tetra- and penta-nucleotides [e.g. $3^4=27$, $4^4=256$] with a few based anchors.

Hanna et al. (2005) well documented the efficiency of ISSR, SSR and SAMPL marker system in detecting genetic polymorphism among 30 winter rye inbred lines and compared the results of cluster analysis performed on data from these marker systems using different statistical methods and coefficients, it was further reported that each marker system was able to discriminate among materials analyzed with lowest value of average genetic similarity (GS) obtained with ISSR markers (0.288) and highest with SAMPLs (0.538). EST-derived SSR turned out to be less efficient in detecting genetic diversity. The average GS value for combined SSR data was 0.3569. Since correlation between similarity and Cophenetic materials was not obtained with various method systems, suggested that different marker system should be used for genetic diversity study to exploit as many sources of polymorphism as possible.

Sarla et al. (2003) reported the discrimination of geographically diverse Oryza nivara accessions and established genetic relationship using ISSR and SSR markers based on AG and GA repeats. Genetic diversity among 24 accession of O. nivara from 11 states of India and five O. sativa varieties, one each from Glasszmniris isozyme group I, II, V and VI were analyzed using ISSR and SSR – PCR. The primers based on AG and GA repeats were informative; their resolving power ranged from 4.2 to 10.8 and PIC from 0.64 to 0.89. They could together enable grouping of accessions on a geographical basis. Ten alleles out of 40 amplified at 6 loci were unique to an accession. Two accessions each from UP and Bihar and one from M.P. were distinct from other accessions. O. nivara
alleles are common with Jaya, Pular, Basmati 370 and Taipei -309 were identified.

Sowframainien et al. (2002) studied the genetic variation in 12 gamma-ray induced mutants in black gram by using RAPD and ISSR marker. In total 35 RAPD and 8 ISSR primers were used and polymorphism was detected. The percentage of polymorphism ranged from 12.5 to 50 for RAPD and 12.5 to 44.4 for ISSR. A significant DNA polymorphism among the mutants were observed using RAPD (28.8) and ISSR (33.3%) markers. A young leaf Chlorina mutant and a smooth pod mutant showed more DNA polymorphism as compared to the parent.

Among the various molecular markers, ISSR profiling is one of the most reliable tools extensively used is many crops plants to work out genetic relationship like Citrus (Fang and Roose, 1997). Vijayan and Chatterjee (2003) selected 11 mulberry (Morus spp.) cultivars, collected from wide range of agro-climatic conditions. ISSR marker system was selected for establishment of genetic relationship and association of marker with leaf yield. The genetic distance among the cultivars varied from a minimum of 0.053, between Punjab local and Bombay local to a maximum of 0.431 between Almora local and Sujanpur-5, three main clusters were obtained. The north Indian cultivars made a separate and distinct group while cultivars originated from eastern and southern India occupied a distinct position. Further, two markers (825.14 and 835.75) associated with leaf yield were also identified.

The microsatellite sequence based marker systems i.e., ISSR and SSR are known to be attractive tool for number of approaches including genetic diversity analysis due to their multiallelic nature, high reproducibility and locus specificity. Previous sequence information is required for the development of SSR primers, which is a major drawback to their application in species, hence studied less intensively. ISSR technique in which polymorphism results from length
differences between inversely oriented closely spaced micro satellites is a co-
effective alternative of SSR.

For many forage crops, characterization of varieties is often difficult due
to a lack of reliable morphological traits and a high degree of intra-varietals
variations. Isozyme markers have limited utility. Moreover molecular markers
(RFLP, RAPD and AFLP) for forage in turf species do not seem convenient for
routine description of varieties. It is the same for microsatellites, which are likely
the most interesting markers, but their development is expensive and at present
few sequences are publicity accessible (Kubik et al., 2001; Jones et al., 2001).

STS marker system:

The good level of polymorphism of STS marker was shown by many studies. The
studies of Bert et al. (1999) and Jones et al. (2002) have shown that the STS
markers can be useful in genetic map construction. However, fodder grasses have
been less intensively studied than other members of Poaceae and few DNA
sequences are developed in the data base.

Patricia and Lallemand (2003) developed 28 STS markers from *Lolium*
sequences especially using consensus sequences from related species of
Gramineae. Primer pairs were designed in order to amplify the intronic regions
thus, the polymorphism detected was based on intronic length polymorphism out
of 42 STS markers development 85.8% yielded successful amplification and 62%
revealed a high level of polymorphism. The analysis of amplicons revealed a
high STS marker specificity, more over the majority of the STS markers can be
considered as “universal markers” because 81% of these STS markers amplified
successfully across 20 related grass species.

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Sethy et al. (2003) reported the isolation of microsatellite sequences and
their conversion to sequence tagged microsatellite sites (STMS) markers in
chickpea (*Cicer arietinum L.*). 10 STMS primer pairs were utilized to analyze the
genetic polymorphisms in 15 *C. arietinum* varieties and two wild varieties. All primer pair amplified polymorphic loci ranging from four to seven alleles per locus with observed heterozygosity ranged from 0 to 0.6667. Most of the STS markers also amplified corresponding loci in the wild relative hence these markers will be useful for the evaluation of genetic diversity and molecular mapping in chickpea.

Beata *et al.* (2003) used 28 STS primers to screen of polymorphisms between two *L. sativus* accessions, ATC 80878 and ATC 80407 resistant and susceptible respectively to *Mycosphaerella pinodes* infection. Ten primer pairs revealed polymorphism between ATC 80878 and ATC 80407 when PCR product were digested with range of endonucleases, results suggested that the STS-based PCR analysis will be useful for generating useful molecular marker in *L. sativus*.

**Application of molecular markers in trait association studies:**

Mishra and Mandi (2004) reported the genetic diversity among 29 Darjeeling-grown tea clones using AFLP marker system. AFLP diversity estimates based on Jaccard’s coefficient allowed separation of the 29 clones into three clusters. Genetic relatedness between the clones was found to be at 70% levels. Further 11 selected clones were used to develop DNA finger prints using 11 random primer which generated 131 polymorphic bands, the activity of drought specific superoxide dismutase (SOD) and ascorbate peroxidase (APX) isozymes was found to be appreciably high in RR17/144, CPI, TV 26 and AV2. The association of specific isozyme activity peak areas a dependent variables and RAPD bands scored was independent variables, stepwise regression showed that the RAPD band (1400bp) obtained with OPAHO2 primer has a highly significant regression coefficient for Cu-Zn SOD activity (*b* = 0.970) and APX 11 activity (*b* = 0.968) using fisher’s exact test (F-test). The association between Cu-Zn SOD and APXII and RAPD band of 1400bp was found to be significant at 99.9% confidence. Being associated with clones exhibiting high activity for drought tolerance
specific isozymes, this DNA band (marker) could be used in germplasm screening for drought tolerance in tea plants.

Nguyen et al. (2004) investigated the influence of genetic background on salt tolerance in Acacia species and also associated RAPD marker with salt tolerance. The seedlings of three provenances form each of Acacia auriculiformis and Acacia mangium were subjected to salt stress for one month. Plant growth, leaf osmotic adjustment and RAPD analysis were studied. In comparison to control plants, the plant growth in all provenances was decreased by salt stress and provenances A3 and M1 were more tolerant than others in A. auriculiformis and A. mangium respectively. Salt stress decreased leaf osmotic potential in all provenances. The difference in osmotic adjustment between the provenances was not correlated with the concentrations of minerals examined such as Na⁺, K⁺, and Ca²⁺ in both species, but it was correlated with the leaf proline concentration in A. auriculiformis. These results suggested that the provenance variation for salt tolerance can be partially accounted for by plant physiological measures. The genetic polymorphism between the provenances was detected by RAPD analysis. Thirty-nine out of 71 bands and 18 out of 63 bands detected were polymorphic for the provenances of A. auriculiformis and A. mangium, respectively. The similarity indices between the studied provenances were less than 65% in A. auriculiformis and 86% in A. mangium suggesting that the provenance variations for salt tolerance were mainly due to the difference in genetic background. The RAPD specific markers for each provenance were determined. These markers can be considered as RAPD markers associated with salt tolerance in the two Acacia species.

Pakniyat et al. (1997) demonstrated the association of salt tolerance characters viz., Na⁺ and carbon-13 with AFLP markers. Out of 204 polymorphic AFLP bands 12 were significantly associated with shoot sodium content and carbon -13 where 6 out of 12 showed the presence of an AFLP band associated with low sodium content and more negative carbon -13 and other six cases these
effects were associated with the absence of an AFLP band. This strategy allows
candidate genetic markers, genotypes and collection sites to be identified for a
suitable trait(s). Forster et al. (2000) reported that fruit weight is a key trait to
successes in tomato salt-tolerance improvement using QTL markers for total fruit
weight under salinity of wild Lycopersicon germplasm (Monfort et al., 1996).
QTL markers related to the drought resistance which are found on chromosomes
of maize (Agrama et al., 1996). Since grasses are generally tough and faces
extreme temperature and other environmental aberrations, it will be interesting to
correlate some of the drought responsive characters namely osmolyte
concentration, proline level, total soluble protein, total sugar and specific leaf area
with the developed DNA patterns. The specific leaf area (SLA) has been found to
be negatively associated with rate of transpiration (TE) which in turn associated
with drought behavior in stylosanthes (Reddy et al., 2000).

Zhou et al. (2006) identified a total of 12 drought related quantitative trait
loci (QTL) by investigating drought tolerance of introgression lines (wild rice)
under 30% PEG treatment at the young seedlings stage. Of these QTLs, the alleles
of 4 QTLs on chromosome 2, 6 and 12 from Dongxiang common wild rice were
responsible for increased drought tolerance of the introgression lines. In
particular, a QTL qSDT12-2, near RM17 on chromosome 12, was consistently
detected in different replications, and expressed stably under PEG stress
throughout the study. It was also found that the QTLs located on different
chromosomes might express at different stages.

**Physiological / biochemical aspects of the plants under water
stress:**

**Osmotic adjustment:**

Cellular responses to water deficit include loss of larger; change is plasma
membrane fluidity and composition, changes in water activity and/or solute
concentration and protein-protein-lipid interaction (Bray, 1997; Heide and Poolman, 2000). Several metabolites that play important role in stabilizing enzyme complex, protecting membranes, and ensuring the osmotic adjustment (OA) required to maintain turgor, are synthesized in response to drought. Osmotic adjustment has been considered as one of the crucial process in plant adaptation to drought stress, because it sustains tissue metabolic activity and enables re-growth upon rehydration but varies greatly among genotypes e.g. it is more important in rice or wheat than in maize (Morgan, 1984). However in term of crop yield there are not many field studies showing a consistent benefit from osmotic adjustment (Quarrie et al., 1999). Osmotic adjustment as normally a slow process and is triggered above a certain threshold of cell water deficit. The osmotic compounds synthesized includes proteins and amino acids (like proline, aspartic acid and glutamic acid) (Samuel et al., 2000; Hamilton and Heckathorn, 2001), methylated quaternary ammonium compounds (e.g., glycine-betaine and alanine-betaine) (Rathinasabapathi et al., 2001; Sakamoto and Murata, 2002), hydorphilic proteins (e.g. late embryogenesis abundant, LEA), carbohydrates like Fructans and Sucrose (Vijn and Smeekens, 1999) and Cyclitons (e.g. D-pinitol, Mannitol) (Anderson and Kohorn, 2001).

The role of organic solute and ion accumulation in osmotic adjustment in drought-stressed grapevines was well documented by Patak et al. (2002), where water relations, gas exchange as well as organic solute and ion accumulation were studied in the leaves of 2-year-old grapevines (Vitis vinifera L., cv. Savatiano) grown under well watered (control) and water stress conditions. Both osmotic potential at full turgor ($\Pi_{100}$) and at turgor loss point ($\Pi_0$) decreased significantly in stressed plants compared with the control. Photosynthetic rate, and stomatal conductance were also significantly lower in stressed plants. Starch concentration decreased almost threefold in stressed plants, while there were no significant differences in sugar accumulation between the two treatments. Total inorganic ion concentration increased rapidly in stressed plants and seems to be the major component of osmotic adjustment in stressed grapevines. Thus, the energetic
cost of osmotic adjustment in grapevines using inorganic ions would be expected to be much lower than for those species using organic solutes (Patakas et al., 2002).

Accumulation of solutes under stress not only decrease cell osmotic potential, but also allowing maintenance of water absorption and cell membrane and metabolic machinery under dehydration. Compatible solutes exert their protective activity by interacting with water molecules, favoring the reduction of solvent. Protein-membrane interaction can lead to the stabilization of protein complex and membranes (Bohnert et al., 1995).

Claudio et al. (2006) reported the divergent selection for osmotic adjustment that resulted in improved drought tolerance in maize (Zea mays L.) in both early growth and flowering phases, the maize (Zea mays L.) cultivars of similar genetic background were taken and results obtained using crops of two S4 populations derived from a cross between inbred lines exhibiting the highest and lowest capacities for osmotic adjustment in a screening applied to 20 inbred lines. The mean values of OA for the two S4 populations were 0.47 MPa for the high OA population (HOA) and 0.06 MPa for the low (LOA). Crops of these populations were grown under a rain-out shelter and subjected to 30-day droughts either before or during flowering. In both experiments, exposure to drought evoked a significant (p < 0.05) decrease in osmotic potential measured at full turgor in the HOA population, no change was found in the LOA population. This induced response became evident in plants of the HOA population in measurements effected 16–18 days after suspension of irrigation. Irrespective of the timing of drought, the HOA crops extracted significantly more water from deeper in the soil profile during the stress period, exhibited higher leaf area duration and attained greater grain yields and, in the crop subjected to water stress at flowering, greater harvest index than the LOA crops. The components of yield and their determinants (i.e., floret number per ear, grain set, grain number and weight per grain) exhibited differential responses with timing of the drought and
in response to level of OA. Under irrigation, there were no differences between populations in either experiment in terms of yield and its components, or in harvest index, leaf area duration, or soil water extraction. This conclude that OA can contribute to drought tolerance in maize crops exposed to water deficit both before and during flowering, and that the trait carries no yield penalty under irrigation.

It should be pointed out that osmoprotection mechanism are not probably functional until severe dehydration occurs with the implication that osmotic adjustment may be critical to survival rather than to increase plant growth and crop yield under drought stress. However, certain reports favor that OA can be related with yield parameters.

Moinuddin et al. (2004) reported the role of osmotic adjustment in drought tolerance of Chickpea (Cicer arietinum L.) and its relation with seed yield and yield parameters. Eight cultivars of differing in OA capacity were field grown. The cultivars were divided into high and low OA groups and crop performance was assessed group wise at three growth stages. As a result, water potential, osmotic potential and RWC decreased progressively with increasing soil moisture stress and age of the crop. As compared to low OA cultivars, high OA cultivars proved significantly superior to low OA ones in seed yield and most of its parameters. The yield and most of its parameters, the yield benefit was 26 and 48% at moderate and severe moisture stress level. This coincided with osmotic adjustment ranging from 0.28 to 0.48 MPa and from 0.37 to 0.71 MPa, respectively at various phases of reproductive additionally, a positive relationship between OA and grain yield in water deficit environment has been shown in grain Sorghum (Ludlow et al., 1990), Basnayake et al., (1995) in wheat (Triticum aestivum L.) (Morgan et al., 1986; Blum et al., 1999), barley (Hordeum Vulgare L.) (Blum, 1989), chickpea (Morgan et al., 1991) and pigeonpea (Cajanuss cajan L.) (Subbarao et al., 2000a).
Many reports regard OA to be causal mechanism favoring crop productivity under water deficit environment. However, there are also some conflicting reports indicating a negative relationship between OA and seed yield under drought (Grumet et al., 1987; Subbarao et al., 2000b). Other reports indicate no relationship between OA and growth and/or seed yield in field conditions under drought (Snackel and Hall, 1983; Munn, 1988; Ludlow, 1986).

Subbarao et al. (2000a) studied the pattern of osmotic adjustment in Pigeonpea (Cajanus cajan L.) for drought tolerance and its effect on yield under drought. Twenty six extra-short-duration pigeonpea [Cajanus cajan (L.) Millsp.] genotypes were grown with irrigation during the growth period or with water deficit imposed from flowering until maturity. Significant genotypic variation was observed in the initiation of OA, the duration of OA and the degree of OA. Based on the measured OA at 72, 82, and 92 days after sowing (DAS), genotypes were grouped into five different clusters. Genotypic differences in total dry matter production under drought were positively associated with OA at 72 DAS ($r^2=0.36^{**}$, $n=26$). Significant positive relationship between OA at 72 DAS and grain yield under drought was found ($r^2=0.16^*; n=26$). However, OA towards the end of pod filling phase, i.e. at 92 DAS, had a significant negative relationship with grain yield under drought ($r^2=0.21^*; n=26$). Genotypic differences in grain yield under drought was best explained using stepwise multiple regression to account for differences in OA at 72, 82, and 92 DAS ($r^2=0.41^{**}; n=78$). The degree of OA at 72 and 82 DAS contributed positively to the grain yield, whereas OA at 92 DAS contributed negatively to this relationship. Since OA is considered as adoptive Mechanism for of plant for drought stress. Need to determine where OA can be used as selection criterion for screening drought resistant cultivars. Jongdee et al. (2002) demonstrated the relationship between OA and low water potential (LWP) in rice, when data were combined across experiments for vegetative and flowering stages. Under water-limited conditions around flowering, grain yield reduction was mainly due to a increased spikelet sterility. Variation in OA was neither related to grain yield nor yield components. There
were however, negative phenotypic and genetic correlations between LWP and percentage spikelet sterility measured at flowering stage on panicles at the same development stage during a water deficit treatment. This suggests that traits contributing to the maintenance of high LWP minimized the effects of water deficit on spikelet sterility and consequently grain yield.

**Protein level under water stress:**

The levels as well as soluble proteins are altered in plants growing under water deficit environment compared with plants growing under non-stressed conditions. Various workers have observed either a decrease (Kumar and Singh, 1991; Hsiao, 1973; Barnett and Naylor, 1966; Yu et al., 1996) or increase (Kumar and Singh, 1991; Rai et al., 1983) in the level of total or soluble proteins in different organs of plants subjected to water stress. The increase or decrease level of protein depends on the plant species and organ studied as well as the severity of the stress. Shah and Loomis (1987) observed decrease content of soluble and total proteins in sugar beet leaves. When Bermuda grass plants were subjected to increasing water stress a decrease in soluble protein level was observed (Barnett and Naylor, 1966). The decrease level of the total soluble protein content in water stressed plants (Kumar and Singh, 1991) appears to be due to more degradation of proteins as well as overall inhibition in protein synthesis under water stress. Genotypes of crop cultivars differing in water stress tolerance, show different levels of total soluble proteins as well as specific activity of protease. Seedlings of drought tolerant mung bean genotypes showed higher protein content as compared with drought sensitive genotypes at -10 barr moisture stress (Kumar and Singh, 1991). Similarly, drought resistant maize (*Zea mays* L.) cultivars show a high protease activity at higher level of water stress, where as inhibition in protease activity in was noticed in sensitive cultivar at same magnitude of water stress (Thakur and Thakur, 1987). While comparing the total protein and free amino acid pool size in drought resistant and drought sensitive cultivars of *C. arietinum* and *Z.mays*, (Rai et al., 1983) observe the resistant plants are characterized by an increase over non-stressed plants in total protein and free
amino acid levels. A genotypes of *C. arietinum* cv C-214 showed an increase of 60% protein over control at an osmotic potential of -3 atm, whereas a sensitive cultivar, G-130, showed a 15% increase over control under similar conditions of water stress. Similarly, drought resistant *Z. mays* cv Ageti-76 plants noticed 190% increase in protein content over control, these observations indicated that that water stress has varying effect on the level of protein in different species, and the stress-induced response depends on the species of crop examined, and it may vary even in different organs within the same species.

**Proline responses:**

One of the most studied compatible solute is amino acid proline. In plants, proline is synthesized in the Cytosol and mitochondria from glutamate via Δ-Pyrroline 5-carboxylate (P5C) by two successive reduction catalyzed by P5C Synthetase (P5CS) and P5C reductase (P5CR) respectively (Hare *et al.*, 1999).

The accumulation of various solutes in the cell to decreases the osmotic pressure of cell for turgor maintenance is known as osmo-regulation sugars and amino acids are major constituents in plants of these, usually there is a remarkable increase in free proline amino acid. Khan *et al.* (1983) studied among free amino acids, proline contributes about 30% fraction of the total amino acid pool. Badhpati *et al.* (1992) reported that proline content increased by 10 to 100 times higher in stressed plants than the non-stressed plants. Moisture stress caused by drought is invariable attended by free proline accumulation in different plant types from bacteria or algae to higher plants. Proline accumulation under stress seems to be depended on light intensity and reserve carbohydrate in plants. Arora and Saradhi (1995) reported the light induced enhancement in proline level in *Vigna radiata* when exposed to environmental stress. Further it was also suggested that photosynthetic activity of seedling might be responsible for the light induced enhancement of proline level. Joyce *et al.* (1992) reported that light enhances the proline accumulation in water stressed leaves of barley and the response was found to be linked with photosynthesis. Talwar *et al.* (2002)
observed and reported the effect of high temperature on proline accumulation, in
different parts and processes of plants i.e., floral parts, invitro pollen germination,
pollen vigour and role of proline in protecting pollen from heat stress in three
groundnut (*Arachis hypogea* L. genotypes, it was concluded that heat injury
during floral development of sensitive genotype may be due to decline in proline
content during early floral development stage and inhibition in the transportation
of proline from author wall to pollen. Tholkappian *et al.* (2001) studied the impact
of water deficit and corresponding change in proline content in mycorrhizal and
non mycorrhizal soyabean, where lower level of proline was recorded in
mycorrhizal soyabean than non mycorrhizal at moisture stress. The nitrogen
content and pod number per plant increased in mycorrhizal soyabean than non-
mycorrhizal plants at 25 percent moisture levels, though accumulation of proline
under stress have been demonstrated in helping plants against desiccation,
increase in proline level have negative effect, suppressing the rubisco activity in
higher plants (Siva kumar *et al.*, 1998).

Francisco (1998) studied the response of 49 Pea cultivars with different
drought tolerance. The tolerance to stress was determined according to the grain
yield or the harvest index in rainfed farming. In these conditions variability
among the genotypes in turgor maintenance, measured as the slope of the turgor
potential (\(\psi_p\)) function against water potential (\(\psi_w\)). The cultivars, which best
maintained turgor, were those which were more drought-tolerant. Turgor
maintenance was significantly related to osmotic adjustment (OA). The free
proline level increased (from 4 to 40 times) in response to water stress. However,
the contribution of this amino acid to \(\psi_{5100}\) was small (approximately 1%) and no
significant relationship was observed between proline content and OA. The
cultivars which accumulated more proline had lower water contents upon turgor
loss. This seems to indicate that proline may play a role in minimizing the damage
caused by dehydration.

Abdel Hamid *et al.* (2003) studied the well--documented effect of
exogenous application proline on the expression of ubiquitin, anti oxidative
enzymes and dehydrins that improve the adaptation of *Pancratium maritium* L to salt stress. The result indicated that the salt stress brought about a reduction of the growth and protein content, particularly at 300mM NaCl. It was significantly increased by exogenous proline, and severely salt stress resulted in an inhibition of the antioxidant enzymes catalase and peroxidase activity, but the activity of these enzymes was maintained significantly in presence of proline.

The role of glycine betaine and proline in improving plant abiotic stress resistance was reviewed by Ashraf and Foolad (2007). Aida *et al.* (2005) studied the over expression of Δ'-Pyrroline-5-Carboxylate synthase increases proline production and confers salt tolerance in transgenic potato.

Proline accumulation appears to be mediated by both ABA- dependent and ABA independent signaling pathways although the events that occur between the perception of stress and induction of proline biosynthetic genes are poorly characterized. Recent evidence supports an important role for post transcriptional event in dehydration and ABA-induced proline synthesizes (Hare *et al.*, 1999).

Accumulation of soluble sugars, free amino acids and proline during stress plays important role in osmotic adjustment in *Sorghum* (Yadav *et al.*, 2002). Increase in the level of solutes and decrease in leaf water potential, solute potential, relative water content (RWC), stomatal conductance and chlorophyll content under stress recovered to normal level after 24 hrs of re-watering the plants.

Claussen (2005) studied the reliability of proline level in measurement of stress in tomato. Tomato (*Lycopersicon esculentum* Mill. cv. Counter) plants were grown in nutrient solutions containing equal nutrient ratios at increasing concentrations (X1 = standard concentration, and X3, X5.5, X8 or X11 = 3, 5.5, 8 or 11 times the standard concentration). Fruit yield decreased significantly from moderate (X3) to high (X11) nutrient concentration during summer, but remained nearly unaffected by the strength of the nutrient solution under low-radiation conditions in autumn. This stress-induced difference in yield was reflected by
higher proline concentrations in leaves of plants grown during the summer compared to those grown during the late season. The decrease in fruit fresh and dry weight observed in summer was due to reduced availability of water and the distribution of dry matter towards the vegetative plant parts at the expense of reproductive growth. The proline content of tomato leaves fluctuated according to nutrient concentration and total radiation, and was closely related to the relative water content of leaves. It was concluded that proline is a reliable indicator of the environmental stress imposed on plants, thus allowing us to establish stress thresholds for fruit yield and product quality of hydroponically grown tomato.

Transgenic tobacco containing osmotin gene induces over expression of proline under both drought and salinity stresses, relative water content and photosynthesis were also seen high in transgenic plant than that of wild type (Gupta et al., 2001). Water stress was found to reduce diurnal leaf water potential and leaf osmotic potential in 60 genotypes but leaf osmotic potential was significantly higher in drought tolerant cultivars C-306 than in the drought sensitive cultivars Kalyansona. Carcellor et al. (1999) studied the water stress around anthesis on proline accumulation and translocation from leaves of two maize cultivars DA 4F37 and DA XL 636) and reported that water stress increased leaf proline content only is DA4F37, while proline in leaf exudates was detected only in DA XL636 water stressed plants. High proline concentration during morning was found in leaves with high relative water content this report also support that proline is involved in osmotic adjustment.

Sunita et al. (1991) reported proline status of genetically stable salt tolerant Brassica juncea L. somaclones and their parent cv. Prakash. The Brassica juncea L. somaclone (SR- 1, 2, 3) selected in vitro for NaCl-tolerance. Non selected somaclones (CP-5) and parent cv. Prakash, and were characterized for their free proline content in absence of stress and as a function at increasing salt stress.
Phutela et al. (2000) studied the water relations, proline content and activities of Pyrroline-5-Carbboxylate Synthetase (PCS) and proline oxidase in five Brassica juncea genotypes under drought tolerance, it was found that drought tolerant cv Varuna showed maximum osmotic adjustment 1/b of 3.21 and proline content increased from 8.4 and 5.3 μ mol/g dry weight under normal conditions to 128.2 and 44.5 μ mol/g dry weight under stress conditions in the leaves and roots respectively. The increase in proline content in less drought tolerant variety Prakash was from 7.0 and 1.8 μ mol/g dry weight in the presence of stress in roots and leaves respectively.

**Enzyme activities:**

Normal metabolism of plant under water stress condition is adversely affected and con-current disturbance of the enzymatic constitution of the plants. Water stress lowers the level of many enzymes in the tissue. (Mali et al., 1980; Geigenberger et al., 1997) water stress leads to oxidative damage in plants by inducing the production of active oxygen species and decreasing the activities of the antioxidant enzyme like catalase, peroxidase and superoxide dismutase (Mali et al., 1980). The magnitude of reactive oxygen species (ROS) production and antioxidant activity decided the tolerance-susceptibility of genotypes (Sairam et al., 2003). The over production of antioxidant enzymes provides an elegance approach to engineer plant species genetically for water stress tolerance. The transformation of alfalfa plants expressing Mn-superoxide dismutase cDNA from Nicotiana spp. have been shown to be more resistant to drought stress (McKersie et al., 1996).

The oxidative burst during which large quantities of reactive oxygen species (ROS) like super oxide, hydrogen peroxide, hydroxyl radicals, peroxide radicals, alkoxy radicals, singlet oxygen etc. are generated in one of the earliest responses of plant cell under various abiotic and biotic stresses and natural course of senescence. The imposition of both abiotic and biotic stress causes over production of ROS, which ultimately impose a secondary oxidative stress in plant.
cell. Degradation of membrane lipids, resulting in accumulation of free fatty acids, initiates oxidative deterioration by providing a substrate for enzyme lipoxygenase, causing membrane lipid peroxidation. Since lipid peroxidation is known to produce alkoxy, peroxy radicals as well as singlet oxygen, these reactions in the membrane are major source of ROS in plant cell. ROS-induced oxidative damage and their protection by anti-oxidative system is discovered, ROS like \( \text{H}_2\text{O}_2 \) acts as a signaling molecule, second messenger, mediated the acquisition of tolerance to both biotic and abiotic stresses (Souman Bhattacharjee, 1995).

Shao et al. (2005) selected 10 kinds of wheat genotypes and reported the anti-oxidative results of maturation stage in terms of activities of POD, SOD, CAT and MDA content as follows: (1) 10 wheat genotypes were grouped into three kinds (A–C, respectively) according to their changing trend of the measured indices; (2) A group performed better resistance drought under the condition of treatment level 1 (appropriate level), whose activities of anti-oxidative enzymes (POD, SOD, CAT) were higher and MDA lower; (3) B group exhibited stronger anti-drought under treatment level 2 (light-stress level), whose activities of anti-oxidative enzymes were higher and MDA lower; (4) C group expressed anti-drought to some extent under treatment level 3 (serious-stress level), whose activities of anti-oxidative enzymes were stronger, MDA lower; (5) these results demonstrated that different wheat genotypes have different physiological mechanisms to adapt themselves to changing drought, whose molecular basis is discrete gene expression profiling. POD, SOD, and CAT activities and MDA content of different wheat genotypes had quite different changing trend at different stages and under different soil water stress conditions, which was linked with their origin of cultivation and individual soil water threshold.

Ismail et al. (2005) investigated the changes in plant growth, relative water content (RWC), stomatal conductance, lipid peroxidation, proline and antioxidant system in relation to the tolerance to polyethylene glycol mediated water stress in drought sensitive common bean \( P. \text{ vulgaris} \) L. accession FM 53
and drought tolerant tepary bean *P. acutifolius* gray accession PI 321–638. For induction of water stress, the 35 days old bean seedlings were subjected to PEG 6000 of osmotic potential −0.40 MPa for 14 days. With regard to vegetative growth, PEG treatment caused more decrease in *P. vulgaris* than in *P. acutifolius* indicating a superior performance of wild species under water stress. Root and shoot DW increased in *P. acutifolius* while decreased in *P. vulgaris* on day 14. PEG treatment had no effects on relative water content (RWC) in *P. acutifolius* but reduced RWC in *P. vulgaris*. *P. acutifolius* maintained a greater stomatal conductance than *P. vulgaris* under water stress imposed by PEG treatment. In *P. acutifolius* constitutive level of lipid peroxidation was lower than in *P. vulgaris* and did not change at the end of the experiment. Constitutive activities of SOD, CAT, APOX and POX were higher in *P. acutifolius* than in the sensitive one and SOD, APOX and GR activities showed an enhancement in the former under water stress. Proline accumulation was also higher in *P. acutifolius* than in *P. vulgaris* both under control and water stress conditions. These results possibly suggest that the drought tolerant tepary bean *P. acutifolius* showed a better protection mechanism against oxidative damage by maintaining higher constitutive and induced activities of antioxidant enzymes, than the sensitive common bean *P. vulgaris*.

A numbers of antioxidant enzymes such as superoxide dismutase, peroxidase and catalase. The superoxide anion, which is the most dangerous reactive oxygen species, is scavenged in plants by superoxide dismutase (SOD EC 1.15.1.1) which converts superoxide anion to hydrogen per oxide. Hydrogen peroxide (H$_2$O$_2$) is scavenged directly by catalase (CAT EC 1:11:1:11) which convert it to water and molecular oxygen peroxides such as ascorbate peroxidase (APX, EC 1.11:1.7) also scavenge H$_2$O$_2$ indirectly by combining it with antioxidant compounds such as ascorbate and guaiacol. Measurement of activities of antioxidant enzymes can be used to indicate oxidative stress in plants.
Isozyme in stress:

Several isozymes like superoxide dismutase, catalase, GR, peroxidase and APX are present in plants and their relative composition changes during exposure to stress (Pinhero et al., 1997). The intensity of many SOD isoforms increased after imposing different stress and it is reported that intensity of SOD-4 isoform was greater than rest of the isoforms after 20 hour of chilling stress in maize (Pinhero et al., 1997). It was reported that esterase and acid peroxidase produced specific and new isozyme bands and the lack of the several isozymes in drought resistant somaclones as compared with the parental cultivars of wheat. Guan and Scandalios (1998) reported that although both sod-4 and SOD-4A transcript accumulates during late embryogenesis, only sod-4 is up regulated by ABA and osmotic stress, it was observed that accumulation of SOD-4 transcript in response to osmotic stress in a consequence of increased endogenous ABA levels in developing embryos. It was also hypothesized that the increase the SOD mRNA in response to ABA is due to in part to ABA metabolic changes leading to changes in oxygen free radicals level, which in turn lead to the induction of the antioxidant defense system. Dubey (1983) reported decrease number of amylase isozyme due to salinity in rice. Mishra et al. (1993) reported that the disappearance of peroxidase isoforms in response to salinity and it was specific to the organ (root and shoot) of tolerant and susceptible lines of green gram. Peroxidase isoforms has been used as sensitive marker for saline stress. Sheoran and Garg, 1997; Chang, 1984) Polymorphism of peroxidase and esterase have been detected in seedlings of the salt sensitive and resistant sunflower varieties, increased activity of peroxidase was also observed in the seedlings of the resistant varieties while it is reduced in the sensitive varieties.