Chapter 4

ROLE OF LIPOXYGENASES DURING GERMINATION AND ABIOTIC STRESS TOLERANCE IN GROUNDNUT
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Lipoxygenases are non-heme iron containing dioxygenases widely distributed in the plant kingdom. They are involved in several plant metabolic processes, such as seed development, germination, vegetative growth, wounding, stress responses, senescence and cell signaling (Porta and Rocha-Sosa, 2002). The multiple functions ascribed to this enzyme are consistent with heterogeneity in plant LOX isozymes, which vary in isoelectric point, pH optima, heat stability, response to Ca\(^{2+}\), and primary product formed (Vick and Zimmerman, 1987). In many plant species, germination is accompanied by elevated levels of LOX activities. Maximal accumulation of LOX protein and the corresponding mRNAs lasts from a few hours to a few days after germination (Porta and Rocha-Sosa, 2002). In the soybean, the best-studied plant with respect to lipoxygenase, two classes of lipoxygenase isoenzymes have been described (Kato et al., 1992a). They have been inferred to play a role in the development of germination capability or in plant defence (Kato et al., 1992b). Analysis of LOX functions and their precise physiological role is complicated by the presence of multiple LOX isozymes in a given plant system.

There is very limited information on the response of LOXs to salt or drought stresses. Bell and Mullet (1991) demonstrated that LOX gene expression in soybean is modulated in plants by water deficit, wounding, and methyl jasmonate. Maccarrone et al. (1995) showed that osmotic stress enhanced LOX-1 and LOX-2 expression without the intermediacy of ABA in soybean. Wounding, MJ or ABA treatment of mature leaves of common bean (*Phaseolus vulgaris* L.) induced LOX
mRNA accumulation suggesting that LOX is required during development and stress conditions (Porta et al., 1999).

Three LOX isozymes have been identified from peanut seed which exhibited differences in pH optima (Sanders et al., 1975; Pattee and Singleton, 1977). Prakash et al. (1990) reported that the LOX activity increased in early stages of germination followed by a decrease in the later course of senescing peanut cotyledons. The LOX activity decreased in peanut cotyledons upon treatment with KN and increased by ABA treatment, chemicals which delay or promote senescence. However, the association of LOX with respect to abiotic stresses has not been studied so far. The present study was aimed at analyzing the LOX activity during germination and in response to NaCl, PEG, ABA and MJ treatments.
RESULTS

4.1 pH optima of lipoxygenases in groundnut seedlings:

As a first step, the optimum pH for groundnut lipoxygenase was determined in 4-day-old seedlings of K-1375 variety. The maximum LOX activity (110 units/mg protein) was recorded at pH 6.5 whereas it was low (12 units/mg protein) at pH 9.0 with linoleic acid as the substrate (Fig. 6).

![Fig. 6: Determination of the optimum pH for 4-day-old germinating seedlings of K-1375 variety of groundnut. The LOX activity in the crude extracts prepared from groundnut seedlings was determined spectrophotometrically at 234 nm by incubating in reaction buffers of different pH (5.0-9.0) with linoleic acid as substrate.]

4.2 LOX activity in germinating seedlings:

LOX activity was estimated during seed germination at 24 h intervals up to 7 days in three varieties of groundnut. LOX activity increased with the onset of germination followed by a marked increase on 4th day in K-1375 and TAG-24 varieties and decreased during subsequent stages of germination. Maximum LOX activity was observed on 5th day in JL-24 variety and decreased thereafter. The levels of LOX activity in 4-day-old seedlings of K-1375 variety were 7 times higher (128 units/mg protein) as compared to one-day-old seedlings (18 units/mg protein). The
LOX activity in 4-day-old seedlings was 6 times higher (90 units/mg protein) as compared to one-day-old seedlings (15 units/mg protein) in TAG-24 variety. The highest LOX activity was observed in 5-day-old seedlings of JL-24 variety which was 5 times higher (75 units/mg protein) when compared to one-day-old seedlings (15 units/mg protein). The highest LOX activities was found in 4-5-day-old seedlings coinciding with the highest growth rate (Fig. 7).

Fig. 7: LOX activity in germinating seedlings of groundnut. The samples were collected at 24 h intervals up to 7 days during seed germination in K-1375, TAG-24 and JL-24 varieties and analyzed for LOX activity spectrophotometrically at 234 nm using linoleic acid as substrate.

The LOX isozyme in mature seeds and 3-6-day-old groundnut seedlings was analyzed using native-PAGE. A single band corresponding to L-3 isozyme was observed in the mature seeds of groundnut. The intensity of this isozyme varied in 3-6-day-old germinating seedlings. Two isozymes viz., L-1 and L-2 were induced in 3-day-old seedlings of K-1375, TAG-24 and JL-24 varieties. In addition to L-1, L-2 and L-3 isozymes, three isozymes (L-4, L-5 and L-6) were induced in 4-day-old seedlings of K-1375 and TAG-24 whereas two isozymes (L-4 and L-5) were induced...
in JL-24 variety. The induction of new isozymes in 4-5-day-old seedlings was associated with a decrease in the intensity of L-3 isozyme (Fig. 8).

Fig. 8: Native PAGE showing LOX isozyme profiles of 3-6-day-old seedlings along with mature seed of groundnut. The crude protein extracts of different samples (20 μg each) were analyzed on native-PAGE and stained with o-dianisidine. Lane 1: Mature seed of K-1375 variety. Lanes 2-4: 3, 4 and 5-day-old germinating seedlings of K-1375 variety. Lanes 5-7: 3, 4 and 5-day-old germinating seedlings of TAG-24 variety. Lanes 8-11: 3, 4, 5 and 6-day-old germinating seedlings of JL-24 variety.

4.3.1 LOX activity in shoots and roots of groundnut seedlings in response to NaCl and PEG treatments:

LOX activity was estimated in roots and shoots of 20-day-old seedlings of K-1375 variety after 24 h of treatment with 200 mM NaCl or 23% PEG. LOX activity increased in roots and shoots in response to NaCl and PEG treatments as compared to controls. However, the increase in LOX activity was more pronounced in roots (68 units/mg protein) than shoots (50 units/mg protein) in response to NaCl treatment as compared to controls. Similarly, a higher LOX activity was observed in roots (77 units/mg protein) compared to shoots (58 units/mg protein) in PEG treated seedlings. It was observed that PEG treatment resulted in higher levels of LOX
activity compared to NaCl treatment. Since the roots exhibited higher LOX activity compared to shoots upon treatment with NaCl and PEG, they were used in subsequent experiments involving different durations of treatment in JL-24 (drought-susceptible) and K-1375 (drought-tolerant) varieties of groundnut (Fig. 9).

Fig. 9: Effect of NaCl and PEG on lipoxygenase activity in roots and shoots of seedlings treated with 200 mM NaCl or 23% PEG. LOX activity was estimated pectrophotometrically in the protein extracts of roots and shoots of 20-day-old seedlings after 48 h of treatment with NaCl or PEG. Values for each treatment are means ± SE of three replicates. Within each group, bars with the same letter are not significantly different (P ≤ 0.05) according to Newman-Keul’s multiples comparisons test.

4.3.2 Time course alteration of lipoxygenase activity in groundnut seedlings in response to NaCl and PEG treatments:

The response of 20-day-old seedlings of JL-24 (drought susceptible variety) and K-1375 variety (drought tolerant variety) after treatment with 200 mM NaCl and 23% PEG was examined. Treatment with NaCl resulted in leaf chlorosis after 24 h in JL-24 variety whereas it occurred after 48 h in K-1375 variety. Leaf chlorosis in PEG treated seedlings was observed after 12 h and 24 h in JL-24 and K-1375 varieties, respectively. Thus the concentrations of NaCl and PEG used in the study caused leaf
chlorosis in both the varieties although the leaf chlorosis was delayed in K-1375 variety compared to JL-24 variety.

LOX activity was determined in roots of 20-day-old seedlings at different time intervals after treatment with 200 mM NaCl in K-1375 and JL-24 varieties of groundnut. Analysis of LOX activity revealed an increase (44 units/mg protein) at 24 h which reached maximum (68 units/mg protein) at 48 h and declined during 72-96 h of treatment in K-1375 variety. In case of JL-24 variety, the maximum activity (30 units/mg protein) was recorded at 24 h and declined thereafter (28-14 units/mg protein). A 2-fold increase in LOX activity was observed at 48 h of treatment in K-1375 variety as compared to controls (Fig. 10a). The LOX activity at 24 h of treatment was found to be 1.6-fold higher than controls in JL-24 variety (Fig. 10b).

Activity staining analysis revealed the presence of three LOX isozymes viz., L-1, L-2 and L-3 in roots of control seedlings of K-1375 and JL-24 varieties. A marked increase in the intensity of L-3 isozyme was observed at 48 h of NaCl treatment followed by a decrease during later intervals in K-1375 variety (Fig. 11). In contrast, the intensity of L-3 isozyme was higher at 24 h and decreased gradually in JL-24 variety (Fig. 12). Thus the increase in LOX activity observed in response to NaCl treatment in K-1375 and JL-24 varieties at specific intervals coincided with the increase in the intensity of L-3 isozyme.
Fig. 10 a & b: LOX activity in roots of groundnut seedlings after treatment with 200 mM NaCl along with controls. The crude protein extracts prepared from roots of 20-day-old groundnut seedlings of K-1375 and JL-24 varieties after 24-96 h of NaCl treatment was assayed for LOX activity spectrophotometrically using linoleic acid as substrate. a. K-1375 variety b. JL-24 variety.
Fig. 11: Native PAGE showing LOX isozyme profiles in roots of 20-day-old seedlings of K-1375 variety after treatment with 200 mM NaCl. The crude protein extracts (20 μg each) of roots of 20-day-old seedlings after 24-96 h of treatment with NaCl were analyzed on native-PAGE. Lanes 1-4, Roots of control seedlings placed in Hoagland’s solution for 24 h, 48 h, 72 h and 96 h, respectively. Lanes 5-8, Roots of seedlings treated with NaCl for different durations of 24 h, 48 h, 72 h and 96 h, respectively.

Fig. 12: Native PAGE showing LOX isozyme profiles in roots of 20-day-old seedlings of JL-24 variety after treatment with 200 mM NaCl. The crude protein extracts (20 μg each) of roots of 20-day-old seedlings after 24-96 h of treatment with NaCl were analyzed on native-PAGE. Lanes 1-4, Roots of control seedlings placed in Hoagland’s solution for 24 h, 48 h, 72 h and 96 h, respectively. Lane 5-8, Roots of seedlings treated with NaCl for different durations of 24 h, 48 h, 72 h and 96 h, respectively.
Treatment of 20-day-old seedlings with PEG resulted in an increase in LOX activity (44 units/mg protein) at 24 h which reached maximum (78 units/mg protein) at 48 h and decreased gradually in K-1375 variety (Fig. 13a). The LOX activity observed at 48 h of treatment with PEG was found to be 2.3-fold higher than controls in K-1375 variety. The LOX activity at 24 h of treatment with PEG was found to be 1.6-fold higher than controls in JL-24 variety (Fig. 13b).

The LOX isozyme profiles in roots of 20-day-old seedlings of K-1375 and JL-24 varieties were analyzed after treatment with PEG. The intensity of L-3 isozyme increased immediately after treatment with PEG followed by a marked increase at 48 h and then decreased subsequently in K-1375 variety (Fig. 14). In case of JL-24 variety, the intensity of L-3 isozyme was higher at 24 h of treatment and thereafter declined (Fig. 15). The increase in LOX activity at 24 h and 48 h of treatment in JL-24 and K-1375 varieties, respectively coincided with the increase in intensity of L-3 isozyme.

In general, the LOX activity induced in response to NaCl and PEG treatments was greater in K-1375 variety as compared to JL-24 variety.
Fig. 13 a & b: LOX activity in groundnut seedlings after treatment with 23% PEG. The crude protein extracts of roots of 20-day-old seedlings of K-1375 and JL-24 varieties after 24-96 h of treatment were analyzed for LOX activity spectrophotometrically using linoleic acid as substrate.

a. K-1375 variety
b. JL-24 variety.
Fig. 14: Native PAGE showing LOX isozyme profiles in roots of 20-day-old seedlings of K-1375 variety after treatment with 23% PEG. The crude protein extracts (20 μg each) of roots of 20-day-old seedlings after 24-96 h of treatment with PEG were analyzed on native-PAGE. Lanes 1-4, Roots of control seedlings placed in Hoagland’s solution for 24 h, 48 h, 72 h and 96 h, respectively. Lanes 5-8, Roots of seedlings treated with PEG for different durations of 24 h, 48 h, 72 h and 96 h, respectively.

Fig. 15: Native PAGE showing LOX isozyme profiles in roots of 20-day-old seedlings of JL-24 variety after treatment with 23% PEG. The crude protein extracts (20 μg each) of roots of 20-day-old seedlings after 24-96 h of treatment with PEG were analyzed on native-PAGE. Lanes 1-4, Roots of control seedlings placed in Hoagland’s solution for 24 h, 48 h, 72 h and 96 h, respectively. Lanes 5-8, Roots of seedlings treated with PEG for different durations of 24 h, 48 h, 72 h and 96 h, respectively.
4.4 Time course alteration of lipoxygenase activity in groundnut seedlings in response to ABA and MJ treatments:

The LOX activity was analyzed at different time intervals in roots of 20-day-old control and treated seedlings of K-1375 variety. A rapid increase in LOX activity was observed at 24 h of treatment with ABA with maximum activity being at 48 h which was 2.5-fold higher than controls (Fig. 16). The LOX activity decreased following 72 h of treatment with ABA although it was higher than controls. A 2-fold increase in LOX activity was observed at 24 h of treatment with MJ compared to control seedlings. The LOX activity decreased with an increase in duration of treatment from 48-96 h (Fig. 17).

Activity staining analysis of LOX isozymes of the roots of 20-day-old seedlings after treatment with ABA and MJ revealed variation in the intensity of L-3 isozyme. The increase in LOX activity observed after treatment with ABA coincided with the increase in intensity of L-3 isozyme as compared to controls. The intensity of L-3 isozyme was higher during 24-72 h of treatment whereas it decreased at 96 h of treatment with ABA (Fig. 18). The marked induction of LOX activity observed at 24 h of treatment with MJ was associated with an increase in the intensity of L-3 isozyme. This isozyme could not be detected at later intervals of treatment (48-96 h) with MJ (Fig. 19).
Fig. 16: LOX activity in groundnut seedlings after different hours of treatment with 100 μM ABA. The crude protein extracts of roots of 20-day-old seedlings of K-1375 variety after 24-96 h of treatment was analyzed for LOX activity spectrophotometrically using linoleic acid as substrate.

Fig. 17: LOX activity in groundnut seedlings after different hours of treatment with 100 μM MJ. The crude protein extracts of roots of 20-day-old seedlings of K-1375 variety after 24-96 h of treatment was analyzed for LOX activity spectrophotometrically using linoleic acid as substrate.
Fig. 18: Native PAGE showing LOX isozyme profiles in roots of 20-day-old seedlings of K-1375 variety after different hours of treatment with 100 μM ABA. The crude protein extracts (20 μg each) of roots of 20-day-old seedlings after 24-96 h of treatment with ABA were analyzed on native-PAGE. Lanes 1-4, Roots of control seedlings placed in Hoagland’s solution for 24 h, 48 h, 72 h and 96 h, respectively. Lanes 5-8, Roots of seedlings treated with ABA for different durations of 24 h, 48 h, 72 h and 96 h, respectively.

Fig. 19: Native PAGE showing LOX isozyme profiles in roots of 20-day-old seedlings of K-1375 variety after different hours of treatment with 100 μM MJ. The crude protein extracts (20 μg each) of roots of 20-day-old seedlings after 24-96 h of treatment with MJ were analyzed on native-PAGE. Lanes 1-4, Roots of control seedlings placed in Hoagland’s solution for 24 h, 48 h, 72 h and 96 h, respectively. Lanes 5-8, Roots of seedlings treated with MJ for different durations of 24 h, 48 h, 72 h and 96 h, respectively.
DISCUSSION

4.5 LOX is induced during germination in groundnut:

The involvement of LOX or the LOX pathway in plant growth and development has been demonstrated in different plant species (Vick and Zimmerman, 1987; Grayburn et al., 1991; Hildebrand et al., 1991). Increase in lipoxygenase activity soon after germination and during early stages of seedling growth has been reported for a number of plant species. In the present study, analysis of LOX activity during seed germination showed a significant increase in 4-5-day-old germinating seedlings coinciding with the maximum growth rate of the tissue. Variation was observed with respect to LOX activity and isozymes in K-1375, TAG-24 and JL-24 varieties of groundnut. The LOX activity increased to 6-7-fold in 4-day-old seedlings of TAG-24 and K-1375 varieties of groundnut whereas a 5-fold increase in LOX activity was observed in 5-day-old seedlings of JL-24 variety. The high LOX activity observed during the early stages of seedling growth suggests that it might be involved in mobilization of storage lipids or has a protective role as the young seedlings are vulnerable to infection by fungal and bacterial pathogens. In rice, total seedling lipoxygenase activity increased by 20-fold, three days after the germination and most of the activity appeared in the developing shoot (Ohta et al., 1986). In corn and sunflower, lipoxygenase activity also increased following germination, but the maximum is not reached until four days after germination (Vick and Zimmerman, 1982). There is also data indicating that LOX is used as temporary storage of nitrogen (Vegetative Storage Protein-94 i.e VSP-94) during vegetative growth.
Weichert et al. (2002) suggested that LOX induced in germinating cucumber seedlings could be involved in biosynthetic activities during germination producing e.g. C6-aldehydes, jasmonic acid or secondary products.

LOXs are multifunctional enzymes as different isozymes perform specific and peculiar functions during various developmental stages. In the present study, analysis of LOX isozymes by activity staining showed the presence of a single lipoxygenase isozyme (L-1) in the mature seeds which was also detected in germinating seedlings. The increase in LOX activity observed in 4-5-day-old seedlings was associated with induction of new isozymes. Three new isozymes, L-4, L-5 and L-6 appeared in seedlings of K-1375 and TAG-24 varieties whereas in JL-24 variety, two new isozymes, L-4 and L-5 were observed. The induction of new isozymes was associated with the decrease in intensity of L-1 isozyme present in mature seeds although their roles in seed germination remain to be investigated. The appearance of new isoforms during seedling growth indicates that seed isozymes and newly synthesized isozymes have different roles. Similarly, new isozymes were induced during germination and seedling growth in pea (Anstis and Friend, 1974; Chateigner et al., 1999), rice (Suzuki and Matsukura, 1997), Arabidopsis thaliana (Melan et al., 1994) and soybean (Kato et al., 1992). In mature soybean seeds, three to four lipoxygenase isozymes were present and during seed germination three additional isozymes appeared in soybean cotyledons (Christopher et al., 1970, 1972; Kato et al., 1992). Mo and Koster (2006) reported that there was a shift of LOX activity during germination from radicles to shoots that accompanies the transition from seed LOXs to vegetative LOXs. Two new vegetative isoforms appeared in the shoots and gradually replaced
the pea seed lipoxygenases as the principal contributors to LOX enzyme activity during early seedling growth.

The involvement of LOX in growth processes is supported by the observation that during germination the new LOX isoforms appeared just before the growth resumption suggesting that these new isoforms have a role in the remodeling of membrane composition during growth (Chateigner et al., 1999). Loiseau et al. (2001) suggested several putative roles for lipoxygenases in seeds such as fatty acid peroxidation in membranes or storage lipids, production of growth regulators (jamonates, abscisic acid), storage proteins and protection against pathogen attack during germination and early seedling growth. On the contrary, Wang et al. (1999) noted that there is no substantial oxygenation of polyunsaturated fatty acids suggesting that LOX is not used for lipid mobilization during germination. Further studies are required for elucidating the role of lipoxygenases induced in germinating seedlings of groundnut.

4.6 LOX induced by NaCl and PEG treatments exhibit a different pattern in drought tolerant and susceptible varieties:

The activation of LOX pathway in plants in response to pathogens, insects or abiotic stresses and at distinct stages of development has been demonstrated (Feussner and Wasternack, 2002). The role of LOX in abiotic stress and induction of vegetative lipoxygenase by water stress has been reported earlier (Mason and Mullet, 1990; Maccarrone et al., 1992; Todd et al., 1992). In the present study, LOX activity in the drought tolerant, K-1375 variety was much higher than that in the drought susceptible JL-24 variety at all the periods of germination. An increase in LOX activity at specific time points was observed in groundnut seedlings subjected to NaCl and PEG
treatments, which was more pronounced in the roots compared to shoots. The pattern of LOX induction varied in susceptible and tolerant varieties. A greater increase in LOX activity was observed in roots of drought tolerant variety in response to NaCl and PEG treatments as compared to drought susceptible variety. LOX activity was found to be 2 times higher in roots at 48 h of treatment with NaCl as compared to controls in K-1375 variety. Similarly, treatment with PEG resulted in an increase in LOX activity by 2.3 times as compared to controls in K-1375 variety. In contrast, maximum LOX activity was detected in roots of JL-24 variety at 24 h of treatment with NaCl and PEG and thereafter decreased. However, the level of LOX activity recorded at 24 h of treatment with NaCl and PEG in JL-24 variety was lower than that of K-1375 variety. It was further noted that the LOX activity induced in response to NaCl and PEG treatments was higher than that of controls for all the intervals examined in K-1375 variety. On the contrary, LOX activity at 96 h after treatment with NaCl and PEG in JL-24 variety was lower or equivalent to controls.

LOX isozyme profiles of roots of K-1375 and JL-24 varieties after treatment with NaCl and PEG showed a variation in intensity of isozyme L-3. The high LOX activity observed during 48-96 h of treatment with NaCl and PEG was associated with an increase in the intensity of L-3 isozymes in K-1375 variety. Thus the pattern of LOX induction varied with the genotype and the treatment. Ben-Hayyim et al. (2001) reported that LOX was induced very rapidly only in the salt-tolerant cells of *Citrus sinensis* in a transient manner. This increase was not observed in the salt-sensitive cells, suggesting that it does not simply reflect a salt-induced damage caused by the stress, but rather a response that might be related to signaling in the defense mechanism. Furthermore, the induction was specific to salt stress and did not occur
with other osmotic-stress inducing agents such as PEG or mannitol, or under hot or cold conditions or in the presence of abscisic acid. These results are in contrast to present observations where LOX was induced in response to NaCl and PEG treatments although differential pattern of induction was observed in K-1375 and JL-24 varieties. The increase in LOX activity observed over time in drought tolerant variety indicates that this enzyme could play an important role during stress tolerance.

4.7 LOX is regulated by ABA and MJ stresses:

The similarities between jasmonic acid and abscisic acid in chemical structure and many physiological effects including plant stress responses are well documented in literature (Weidhase et al., 1987; Sembdner and Parthier, 1993; Melan et al., 1993). Abscisic acid (ABA) and methyl jasmonate (MJ) treatment have been shown to induce LOX expression in different plant species. It was therefore of interest to know whether LOX activity is induced in response to treatment with growth regulators such as MJ and ABA, that have been involved in stress responses. In the present study, treatment of 20-day-old seedlings with ABA resulted in an increase in LOX activity in roots at 24 h which reached maximum levels at 48 h and subsequently decreased. The LOX activity induced in response to ABA treatment remained higher than controls for all the intervals examined. The increase in LOX activity was associated with an increase in the intensity of L-3 isozymes although the intensity of the band varied. These results support the findings of Prakash et al. (1990) who reported that KN treatment lowered the lipoxygenase activity where as ABA treatment increased the LOX activity when compared to untreated seedlings in groundnut. It has been suggested that ABA enhanced lipoxygenase activity could be due to the breakdown of cell membranes in senescing tissues, which releases large quantities of free fatty
acids, especially linoleic acid (Draper, 1969). Porta et al. (1999) showed that LOX mRNA levels were higher in the mature region than in the growing region of bean seedlings subjected to drought or treated with ABA. The contrasting expression pattern was suggested to be the result of a decrease in the seedling growth rate provoked by drought or ABA treatments (Creelman et al., 1990; Colmenoero-Flores et al., 1999).

The present study showed a 2-fold increase in LOX activity at 24 h in groundnut seedlings upon treatment with MJ as compared to controls. The LOX activity drastically reduced at 48 h and reached values lower than control at 96 h of treatment with MJ. The increase in LOX activity at 24 h of treatment with MJ was associated with an increase in intensity of L-3 isozyme. These results suggest that LOX activity is induced transiently by MJ in groundnut. This, however, is different from the sustained induction of LOX observed in groundnut seedlings treated with ABA. The rapid and transient expression of PnLOX1 gene in mature seeds to methyl jasmonate has been reported for peanut (Burow et al., 2000). The expression of wound-inducible maize LOX with dual positional specificity was unusual in that it was expressed in both early and late stages of the stress response in exogenously supplied MJ (Kim et al., 2003). Melan et al. (1993) reported that LOX1 mRNA levels were elevated in Arabidopsis roots treated with MJ within 24 h and remained above control levels for at least 96 h.

The present experiments involving NaCl, PEG, ABA, and MJ showed a clear increase in the intensity of L-3 isozyme although variation was noticed with respect to the intensity of isozyme and timing of induction. Since the pattern of LOX induction after treatment with PEG was more similar with that of ABA, it is possible that ABA
acts as a mediator in this response. Tamas et al. (2009) hypothesized that the role of LOX in plant metabolic processes in the roots may depend on the level of reactive oxygen species: at physiological concentrations of ROS, LOX may be involved in the processes of root growth, while at the elevated concentrations of ROS induced by different stress conditions, it may be involved in root growth inhibition through ecotopic differentiation.

In summary, the studies showed a maximum induction of LOX activity in 4-5-day-old seedling along with induction of additional isozymes in groundnut. The pattern of LOX induction varied in JL-24 (drought susceptible) and K-1375 (drought tolerant) varieties in response to treatments with NaCl and PEG with the maximum increase being observed in K-1375 variety compared to JL-24 variety. Similarly, treatment with ABA and MJ resulted in an increase in LOX activity in K-1375 variety, but the pattern of LOX induction was sustained with ABA compared to transient induction with MJ. These experiments provided preliminary evidence of the involvement of LOX in tolerance to abiotic stresses in groundnut.