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SHORT COMMUNICATION

IN VITRO EVALUATION OF RELATIVE TOLERANCE OF *ELEUCINE CORACANA* GENOTYPES AGAINST WATER DEFICIT STRESS

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Finger millet (*Eleusine coracana* L.) is considered a drought-tolerant crop . However, within this species, there is considerable genotypic variation in tolerance to this environmental stress. In the present work, effect of mannitol -induced water deficit stress on seed germination, early seedling growth, proline, MDA and hydrogen peroxide content in ten finger millet genotypes were analyzed. The seedlings of PRM-6107, VL-283 and VL-328 recorded relatively better drought tolerance in terms of growth and oxidative damage . While, PES-400, VR-708, VL-149 and VL-146 showed susceptibility as per cent reduction in germination, shoot growth, root growth, MDA content and hydrogen peroxide level was relatively higher in comparison to tolerant group. Rest of the three genotypes viz. VL-315, PR-202 and PRM-9802 showed intermediary character in between these two groups.

***Key words*: *Eleusine coracana*, finger millet, hydrogen peroxide, proline, ragi, water deficit stress.**

Plants differ in their resistance to water deficit stress because of differences in morphological, physiological, biochemical and molecular adaptive mechanisms. Genetic differences in water deficit stress tolerance offer the unique opportunity to compare the changes in metabolic processes in plants under water stress that might be involved in water deficit stress tolerance. Genotypic diversity existing in finger millet is well known and hence is the primary reason that holds promising for further improvement for water deficit tolerance. One approach in studying relative tolerance of genotypes against water deficit is to measure growth accompanied by biochemical changes under stress condition. Use of per cent germination and plant growth parameters as a selection index for screening of water deficit tolerant genotype has been described in barley (Laszczynska 1991), legume plants (Grzesiak *et al*. 1996) and sugar beet (Sadeghian and Yavari 2004) etc. There are numerous reports of the accumulation of organic solutes in plants under water-stress induced by dehydration or hyperosmotic conditions (Stewart and Larher 1980, Edwards *et al*. 1988, Vance and Zaerr 1990). The possibility of using proline content as a selection trait in breeding for drought tolerant genotype has been reported by several workers (Singh *et al.* 1974, Rajgopal *et al*. 1977). Hydrogen peroxide is a good biochemical indicator of damage associated with water deficit stress (Sharma and Dubey 2005). Several investigators considered malondialdehyde (MDA) content, a product of lipid peroxidation as an indicator of oxidative damage. Thereby, cell membrane stability has widely been utilized to differentiate drought-tolerant and drought sensitive cultivars (Zhang and Kirkham 1994, Guo *et al.* 2006). Studies related to the use of growth and biochemical parameters for evaluation of water deficit tolerance under *in vitro* condition are scarce in finger millet. In the present study we describe the

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differences in growth and biochemical responses of ten finger millet genotypes under mannitol-induced water deficit stress.

Seeds of seven genotypes viz. VL-315, VR-708, VL-149, VL-146, PR-202, VL-283 &VL-328 and three genotypes viz. PES-400, PRM-6107 & PRM-9802 were obtained from Vivekanand Parwatiya Krishi Anusandhan Sansthan (VPKAS), Almora and Hill Campus, G.B.Pant University of Agriculture and Technology, Ranichauri, respectively. After surface sterilization, these seeds were placed on solidified MS (Murashige and Skoog 1962) medium supplemented with different mannitol concentration (0, 200, 400 mM) at 26±1ºC temperature, 70% humidity and photon flux density of approximately 60 µmol $m^{-2}s^{-1}$ with a 16/8hrs day/night cycle. The emergence of roots through the seed coat, was determined for total number of seeds sown per treatment on 4th day. After eight days, length of shoots and roots were measured from three replicates.

Free proline was determined from 8-day-old-seedling by the method of Bates *et al*. 1973. Hydrogen peroxide from 8-day-old seedling was measured spectrophotometrically after reaction with potassium iodide (Alexieva *et al*. 2001). Lipid peroxidation was determined by measuring the amount of MDA produced by the thiobarbituric acid reaction as described by Heath and Packer (1968). For each extract, the absorbance was determined on duplicate assays. The standard deviations are given with means.

Responses of ten genotypes were observed for relative tolerance to mannitol -imposed water deficit and six parameters were analysed for this study (Figs.1-6). All the parameters studied differ significantly among genotypes as well as between treatments. In all the genotypes tested, seed germination was reduced as the mannitol concentration increased from 0 to 400 mM (Fig. 1). Generally, in control plants of all the genotypes germination of 38.8% to 50.1% was observed on 4th day except for PRM-9802 which showed 28.3% germination. However differential response of ragi genotype was observed at 200 mM and 400 mM mannitol concentration; for example, out of ten, five ragi genotypes viz. VL-315, VL-283, PR-202, PRM-6107 and VL-328 showed 11.37 to 14.28% reduction in per cent seed germination at 200 mM mannitol concentration over controls, where as the values for PES-400, VR-708, VL-149 and VL-146 in the same treatment were ranged from 19.9 to 27.7%. Similarly, at 400 mM concentration, VL-315, VL-283, PR-202, PRM-6107 and VL-328 showed 19.04 to 23.5% reduction in per cent seed germination over controls, where as the values for PES-400, VR-708, VL-149 and VL-146 were 31.7 to 42.5%. At both the stress level PRM-9802 showed highest reduction in per cent seed germination *i.e.* 37.6% (200 mM) 56% (400 mM) over control.

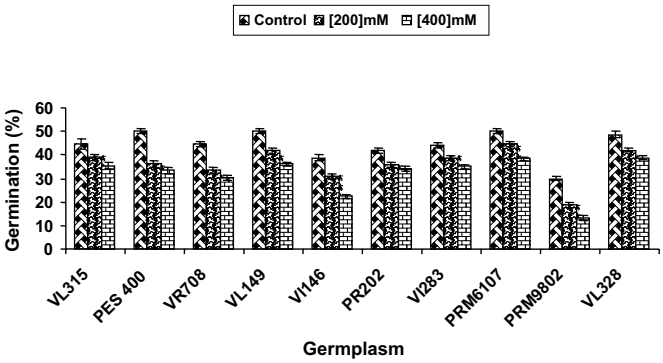

Fig. 1. Effect of mannitol -induced water deficit on per cent germination of *E.coracana germplasm(* genotypes). n = 4 ± SD.

Seedling growth in terms of shoot length (Fig. 2) and root length (Fig. 3) was also affected under stress. Shoot growth and root growth were negatively affected by an increase in mannitol concentration. Differences in shoot and root growth among genotypes were observed. At 200 mM mannitol concentration, the decrease in shoot and root length of the four out of ten genotypes viz. PES-400, VR-708, VL-149 and VL-146 was comparatively

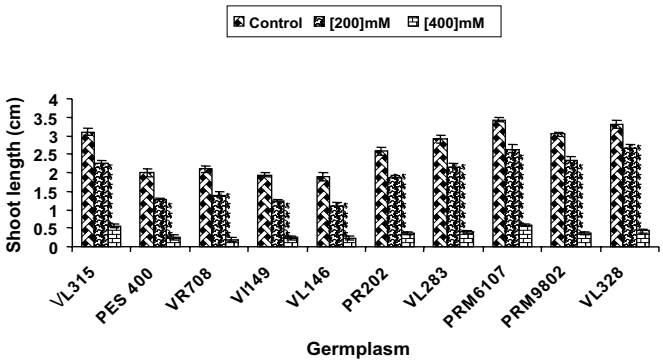

Fig. 2. Effect of mannitol -induced water deficit on shoot length of *E.coracana* seedlings. n = 3 ± SD.

higher than the decrease in shoot length of the another six genotypes namely VL-315, PR-202, VL-283, PRM-6107, PRM-9802 and VL-328. At 400 mM mannitol, shoot and root growth were severely affected.

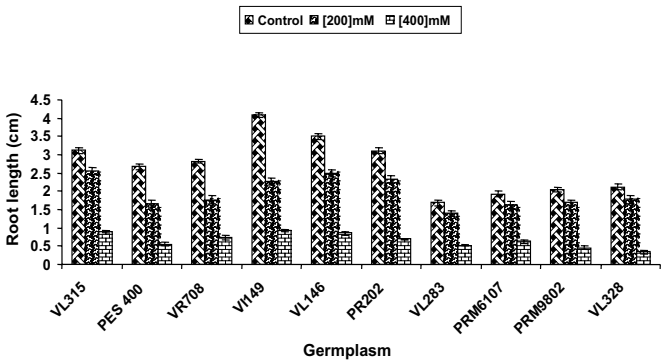

Fig. 3. Effect of mannitol -induced water deficit on root length of *E.coracana* seedlings. n = 3 ± SD.

Seed germination and plant growth were used as the criteria for water deficit tolerance. In this case, with the exception of PR-202 and PRM-9802, responses of all the genotypes measured by germination rate were correlated with those measured by growth. However, PRM-9802 despite of the highest reduction in per cent germination showed relatively lesser reduction in root growth. Similarly, PR-202 showed relatively lower reduction in per cent germination but higher reduction in root length. Hurkman and Tanaka (1989), working with barley, reported that varietal differences in salt tolerance measured by germination rate may differ from those measured by plant growth. Several investigators have reported positive relationship between plant growth and per cent germination with drought tolerance (Grzesiak *et al*. 1996, Sadeghian and Yavari 2004). Decrease in per cent germination of seeds under stress is a well documented phenomenon (Seeman and Critchly 1985). On the basis of germination and growth study, PES-400, VR-708, VL-149 and VL-146 emerged as more susceptible genotypes while VL-315, VL-283, PRM-6107 and VL-328 emerged as less susceptible genotypes. However, in case of PRM-9802 and PR-202 negative relationship was found between plant growth and per cent germination. These two groups were further explored for their relatively less or more susceptible mechanism. In order, to measure oxidative damage due to mannitol- induced water deficit hydrogen peroxide content and lipid peroxidation were measured. In all the genotypes tested, hydrogen peroxide (Fig. 4) content increased as the mannitol concentration increased from 0 to 400 mM. However, higher hydrogen peroxide level at 400 mM mannitol concentration (569.3 to 1424.4% increase over control) in leaves of PES-400, VR-708, VL-149 and VL-146 showing its susceptibility. While, in case of other group (VL-283, PRM-6107 and VL-328) only 190.3% to 288.3% increase was observed with the exception of VL-315 which showed 863.09% increase. Other two, genotypes i.e. PR-202 and PRM-9802 showed 473.9% and 419.7% increase over control.

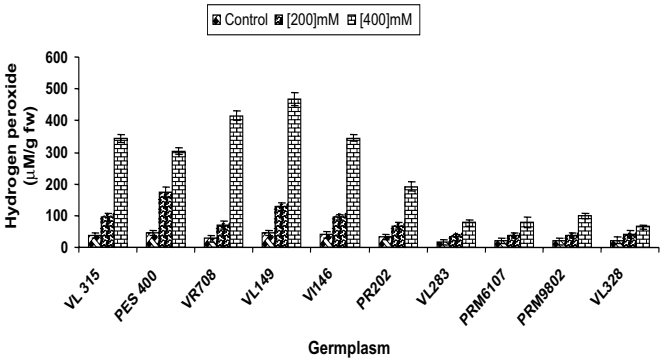

Fig. 4. Changes in hydrogen peroxide content of 8-day-old seedlings of *E. coracana* germplasm (genotypes) under different level of mannitol -induced water deficit stress. n= 3 ± SD.

Lipid peroxidation in leaves of ten *E.coracana* genotypes, measured as MDA content, is given in Fig. 5. It shows that significant differences in MDA content were found among genotypes as well as between treatments. Generally, MDA content in control plants ranged from 0.9-1.37 µmol/g.f.wt. Susceptible genotypes i.e. PES-400, VR-708, VL-149, VL-146 have significantly higher MDA content while in relatively less susceptible genotypes *i.e.*VL-283, PRM-6107, and VL-328 MDA content was not affected by mannitol-induced water deficit stress. In case of PRM-9802 our result of peroxide content was in agreement with lipid peroxidation as it showed only 9.6% increase in MDA content over control. Similarly in case of VL-315 and PR-202 result of peroxide content was in agreement with lipid peroxidation as both genotypes showed relatively higher MDA content, i.e. 93.37% and 66.1%, respectively. An increase in hydrogen peroxide level and MDA content

was detected in rice cultivars under drought condition (Guo *et al*. 2006). They also found positive correlation between hydrogen peroxide content and MDA content. On the basis of measurement of oxidative damage in terms of hydrogen peroxide and MDA content previously categorized less susceptible group was emerged as relatively tolerant group with the exception of VL-315. Though, VL-315 showed better growth but they don't record lower oxidative damage.

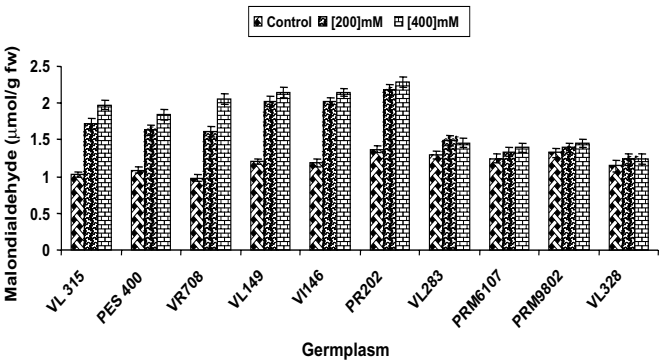

Fig. 5. Changes in malondialdehyde content of 8-day-old seedlings of *E. coracana* germplasm (genotypes) under different level of mannitol -induced water deficit stress. n= 3 ± SD.

Further, we identified proline content in all the genotypes to evaluate their adaptation response. The results (Fig. 6) revealed, in relatively tolerant group PRM-6107 and VL-283 showed ~32-fold increase over control as compared to VL-328 which showed ~28- fold increase over control at 400mM concentration of mannitol. This was followed by PRM-9802 and PR-202 showing ~27-fold increase over control for the same treatment. However, genotypes categorized as susceptible namely PES-400, VR-708, VL-149 and VL-146 showed ~18-22- fold increase at 400mM mannitol concentration. Interestingly despite of the better growth VL-315 showed relatively lesser increase i.e. ~16-fold in proline content. Delauney and Verma (1993), Singh *et al*. (1973), Mali and Mehta (1977) and Sivaramkrishnan *et al*. (1988) correlated proline accumulation with water deficit stress tolerance. However, Hanson *et al*. (1979) and Ilahi and Dorffling (1982) reported higher proline accumulation in susceptible cultivars. Our results revealed relatively lower content in susceptible group as compared to relatively tolerant group. In view of these conflicting reports it is difficult to conclude whether high proline accumulation is associated with drought-susceptible or drought-tolerant nature of cultivars.. It is also not clear whether proline accumulation is an adaptive response to water stress or biochemical change consequent upon injury. Future studies are required in this regard.

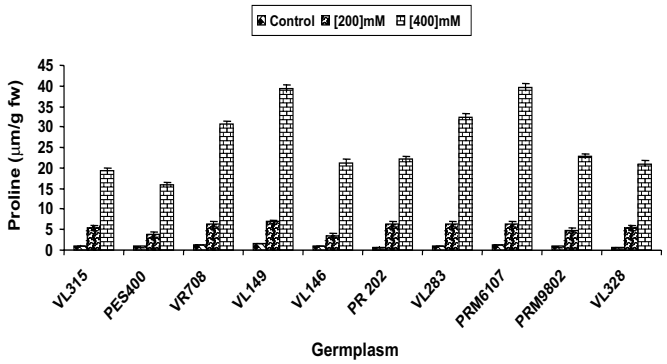

Fig. 6. Changes in proline content of 8-day-old seedlings of *E.coracana germplasm* (genotypes) under different level of mannitol -induced water deficit stress. n = 3 ± SD.

Our data indicates that PRM-6107, VL-283 and VL-328 were relatively tolerant as better growth accompanied by lower oxidative damage had shown by these genotypes under mannitol- induced water deficit stress. While, PES-400, VR-708, VL-149 and VL-146 showed susceptibility as relatively poor growth and higher oxidative damage were observed in these genotypes under same condition. Rest of the three genotypes namely PRM-9802, PR-202 and VL-315 could not be placed under tolerant or susceptible group because they were showing intermediary character between these two groups.

This study revealed a different ranking compared to what would have been expected from the results of previous studies. However, previous studies regarding water deficit tolerance associated behavior of all the mentioned genotypes were not available. Sarlach and Gill (1992) have reported PR-202 as a sensitive genotype on the basis of biochemical changes in germinating seeds under PEG- induced water deficit stress. However, we observed PR-202 as moderately tolerant genotype. In some of the previous studies, highest photosynthesis followed by highest transpiration rate was observed in

the genotype, VL-283 (Anonymous, 2007). This data is very close to our findings as we have ranked VL-283 as tolerant genotype on the basis of growth and oxidative damage. It may be concluded that growth behavior and oxidative damage in the *in vitro* assays may be used as an early selection tool for relative tolerance against water deficit stress.

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