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Extraction and characterization of Pearl Millet Lipids and changes in enzyme activities in developing grains under water deficit and irrigated conditions

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Abstract

Pearl millet is a high-vitality oat that contains starches, protein, and fat, wealthy in nutrients B and A, high in calcium, iron, and zinc, and furthermore contains potassium, phosphorus, magnesium, zinc, copper, and manganese. India is the biggest maker of pearl millet. It is a yearly grass, erect and coming to up to 3 m high with a plentiful root framework. An endeavor was made to ponder its enzymatic exercises under various conditions. The present examination was made to comprehend the conduct of pearl millet lipid submerged shortfall and irrigated condition.

Keywords: Cereal, iron, fat, water deficiency

Introduction

Food security has consistently been key need because of expanding interest for sustenance with regularly expanding populace. Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a significant coarse grain oat and scavenge harvest of the dry and semi-parched tropics of the Indian subcontinent and a few African districts. It is a focal part of the nourishment and grain security of the rustic poor dwelling in these regions. Pearl millet generation is gathered in the creating nations which record for more than 95 % of the creation and land (Basavaraj *et al.* 2010) ^[1]. In India, pearl millet was become over a zone of 7.9 and 7.1 million hectares with all out creation of 9.2 and 9.1 million tons every year and profitability of 1161 and 1272 kg/ha during 2014 and 2015, separately (Anonymous 2014; Anonymous 2015). Among the ranchers half breeds are more mainstream than the composite assortments as a result of higher yield. Pearl millet is a tropical plant having the C4 photosynthetic pathway with resilience to dry season, warmth, and low soil pH (Maiti and Wesche-Ebeling 1997) ^[2]. The pearl millet grain includes around 8 % pericarp, 17 % germ (which is relatively enormous) and 75 % endosperm (Serna-Saldivar and Rooney 1995) ^[3]. Wheat is hard external layer of the grain which comprises of joined aleurone, pericarp and part of germ. Nourishing profile of pearl millet is superior to numerous different oats. Pearl millet is the least expensive wellspring of vitality, protein, Fe and Zn among all grains and heartbeats (Rai *et al.* 2013) ^[4]. Grain protein substance of pearl millet half and halves and assortments discharged in India runs between 8.00 to 13.00 % (Anonymous 2013). Pearl millet is additionally a decent wellspring of lipids. Lipids substance ranges from 4.1 to 7.5 % (Goyal *et al.* 2014) ^[5]. Most oat grains don't contain enough oil to be viewed as appropriate for business oil creation, yet as referenced above pearl millet is likewise a decent wellspring of lipids. Omega 3 and linolenic corrosive involves about 4% of the all out unsaturated fats present in pearl millet lipids (Burton *et al.* 1972) ^[6] giving it a higher substance of n-3 unsaturated fats than other oat grains. In the meantime nearness of high measure of lipids in pearl millet additionally is one of the reasons for poor storable existence of its essential items for example flour and grain. In any case creating pearl millet cultivars with higher substance of lipids in perspective on their healthful significance are not unfortunate. The advancement of off-season in pearl millet flour upon capacity is an old and uncertain issue related with pearl millet and is the significant obstacle for its customer adequacy. The supper builds up a drab acidic smell inside a couple of hours in the wake of granulating (Varriano-Marston and Hosenev 1983) ^[7]. Storability of pearl millet flour is basically subject to lipids content (Kaced *et al.* 1984) ^[8] and developed of fat corrosiveness (FA) (Lai and Varriano-Marston 1980b) through hydrolytic cleavage of triglycerides by the activity of lipase (Kaced *et al.* 1984) ^[8]. The present examination entitled, "Biochemical Studies on Synthesis, Hydrolysis, Characterization and Storage of Pearl Millet Lipids" was completed in the Department of Chemistry and Biochemistry in a joint effort with Bajra Section, Department of

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Genetics and Plant Breeding, CCS HAU, Hisar with the point of Profiling amassing of lipids and changes in catalyst exercises in creating grains submerged deficiency and inundated conditions

Materials and Methods

Raising of pearl millet crop under irrigated and water-deficit conditions for comparing pattern of changes in activities of enzymes in flag leaf and accumulation of nutrients in developing grains Pearl millet hybrid HHB 94 was grown in two sets each consisting of 10 rows with 10 cm intra row and 45 cm inter row spacing at Research farm of Bajra Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar during *kharij*-2014. The crop was sown in the month of August, 2014 following the recommended package of practices. Both set of plants were irrigated twice till heading. Afterwards one set of plants was irrigated regularly and no further irrigation was given to another set to maintain water-deficit condition. Total rainfall received during the period preceding the onset of anthesis was 81.5 mm and 20.3 mm during the post anthesis period.

Collection of flag leaf and developing grain samples for analysis

Whole plant was uprooted on every fourth day starting 0 DAA (day after anthesis) till 32 DAA and brought to the lab. Flag leaf was removed from main tiller of the plants. Excluding the mid rib, middle portion of the leaf (leaving 5 cm portion each from top and bottom) was used immediately for making extract for assaying enzymes. Simultaneously the corresponding ear head on the main tiller was also removed. After drying of the ear head under shade, the same was sun dried for several days before removing the grains. Grains were removed by manual abrasion. After recording test weight these were stored at -20°C till further use for chemical analysis.

Chemicals

All the chemicals used during the present course of investigation were purchased from either of the followings: Sigma Aldrich Chemical Company (St. Louis, M.D., USA); Himedia Laboratories Limited, Bombay; Sisco Research Laboratories Pvt. Limited, Bombay; SD Fine-Chem. Ltd., Mumbai, Loba Chemie Pvt. Ltd, Mumbai and Merk Millipore, Mumbai. All the chemicals were of the analytical grade.

Extraction of crude lipids

Free lipids from flour or bran were extracted in petroleum ether (60-80°C) by using SOCS PLUS system (Pelican Equipments, Chennai). Method of AOAC (1990) with slight modifications was used to determine crude lipids content (%). Five gram of dry flour was taken in a nitrocellulose thimble of 25 mm x 80 mm size (Whatman, England) fixed onto a thimble holder. The thimble fixed on to the holder was suspended in pre-weighed extraction beaker containing

approximately 100 ml petroleum ether. The extraction beaker holding the thimble was then kept on the hot plate of the equipment maintained at 180°C . The beaker was then fixed with the collecting vessel ensuring a proper connection between these. For complete extraction of fat, the process was carried out for at least 40 minutes. Level of the extraction solvent was maintained by putting extra solvent from small funnels connected through a silicone rubber seal fixed on the top of the collecting vessel so that during the extraction period whole of the flour in the thimble was continuously in contact with the solvent. Extraction was stopped by blocking the flow of petroleum ether from collecting vessel to the extraction beaker by closing the valve. The excessive solvent in the extraction beaker was allowed to evaporate and was collected in the collecting vessel. Then extraction beaker containing oil and small amount of petroleum ether was removed from the hot plate and transferred into an oven maintained at 50°C for evaporating traces of the solvent. The beaker containing oil was weighed. The empty thimble after proper cleaning with a soft brush was reused for the next sample. The amount of oil was calculated by taking difference between weight of the beaker before and after extraction. Results are expressed as per cent of dry flour.

Results and Discussion

Depositions of free lipids and crude protein in developing grains

Deposition of free lipids and protein in developing grains of HHB 94 grown during *kharij*-2014 under irrigated condition as well as water deficit condition on every fourth day starting from 12 DAA till 32 DAA was monitored. Test weight of grains was also recorded. Results are presented in Fig. 1. Per cent free lipids deposited by 12 DAA in developing grains of plant grown under normal irrigated condition and water deficit condition were 7.3 and 6.1. Thus grains of plant raised under water deficit condition had accumulated 1.2 % lesser lipids. With further development of grains of plants raised either under normal irrigated or water deficit condition per cent lipids deposited statistically did not change. Similarly lipids accumulated in mature grains (32 DAA) of plants raised under normal irrigated and water deficit conditions differ only by 1.3 % *i.e.* normal grains had 7.6 whereas water deficit grains has 6.3 per cent lipids. At every stage of development per cent crude protein content, on the other hand, was higher in grains of plants grown under water deficit condition than those grown under normal irrigated condition. For example grains of water deficit and normal irrigated plants had 11.62 and 9.77 per cent protein on 12 DAA. Like lipids, statistically similar concentration of crude protein was present in fully mature grains. Thus per cent lipids in mature grains of plants grown under irrigated condition was higher than those of plants grown under water deficit condition, whereas per cent crude protein was higher in water deficit condition. At every stage of sampling test weight of grains produced from plant grown under water deficit condition was also significantly lower than those of irrigated plants:

Table 1: Crude lipids and protein contents of developing grains of pearl millet hybrid HHB 94 grown under irrigated and water deficit conditions

Day(s) after anthesis	Free lipids (%)		Crude Protein (%)	
	Irrigated	Water deficit	Irrigated	Water deficit
12	7.3 ± 0.24	6.1 ± 0.13	9.77 ± 0.18	11.62 ± 0.03
16	7.5 ± 0.14	6.3 ± 0.09	9.87 ± 0.11	11.88 ± 0.01
20	7.6 ± 0.11	6.4 ± 0.12	9.82 ± 0.09	11.89 ± 0.13
24	7.3 ± 0.13	6.2 ± 0.11	9.90 ± 0.13	11.94 ± 0.05
28	7.4 ± 0.09	6.5 ± 0.20	9.82 ± 0.11	11.72 ± 0.14
32	7.6 ± 0.21	6.3 ± 0.15	9.81 ± 0.09	11.74 ± 0.14

Nitrate reductase (NR)

Profile of change in the activity of NR in flag leaf of plants grown under different conditions is depicted in Fig. 2. At the onset of anthesis NR activity in leaves of plants grown under normal condition was $12.68 \mu \text{ moles NO}_2^- \text{ g}^{-1} \text{ h}^{-1}$ that declined sharply to $4.74 \mu \text{ moles NO}_2^- \text{ g}^{-1} \text{ h}^{-1}$ till 12 DAA and slowly afterwards till 28 DAA. By 28 DAA activity fell to a minimum level of $2.69 \mu \text{ moles NO}_2^- \text{ g}^{-1} \text{ h}^{-1}$. Profile of change in activity of the enzyme of the plant grown under water deficit condition was similar to those grown under normal irrigated condition. Thus effect of environment was more pronounced during early stages of plant growth (till 8DAA). As the plants attained maturity the enzyme showed almost same activity under two different environments. NR activity of plants grown under water deficit condition was lower than those grown under normal irrigated condition particularly till 8DAA. For example compared to $11.0 \mu \text{ moles NO}_2^- \text{ g}^{-1} \text{ h}^{-1}$ of activity on 4DAA of plants grown under irrigated condition only $6.00 \mu \text{ moles NO}_2^- \text{ g}^{-1} \text{ h}^{-1}$ of activity was present in the plants grown under water deficit conditions.

Nitrite reductase (NiR)

Qualitatively pattern of change in the activity of NiR in flag leaf of the plants raised under two different conditions was similar to that of NR activity, however, activity decreased almost linearly with advancement of growth till 20 DAA. Thereafter activity did not change till maturity of the plants. Magnitude of NiR activity assayed from the samples grown under two different environments was significantly different. As shown in Fig.3 activity under irrigated condition declined from 221.7 to $39.1 \mu \text{ moles NO}_2^- \text{ g}^{-1} \text{ h}^{-1}$ between 0 and 28 DAA and under water deficit condition activity declined from 198.5 to $16.1 \mu \text{ moles NO}_2^- \text{ g}^{-1} \text{ h}^{-1}$ during the same period of time.

Glutamine synthetase (GS)

Figure 1 Represents changes in the activity of GS in flag leaves of pearl millet genotype grown under normal (irrigated) and water deficit conditions. Enzyme showed the same trend as that of NR and NiR *i.e.* its activity was maximum on 0 DAA and after that it declined along with growth of the plants. At 0 DAA activity of this enzyme in the plants grown under normal and water deficit conditions was 965.3 and $861.1 \mu \text{ moles } \gamma\text{-GMH g}^{-1} \text{ h}^{-1}$, respectively. Under irrigated condition activity declined from 965.3 to $743.5 \mu \text{ moles } \gamma\text{-GMH g}^{-1} \text{ h}^{-1}$ between 0 and 8 DAA. Similarly under water deficit condition the activity declined from 861.1 to $704.5 \mu \text{ moles } \gamma\text{-GMH g}^{-1} \text{ h}^{-1}$ during corresponding period of time (Fig 4). Enzyme activity declined continuously in both kinds of plants till 16 DAA. By this day the plants has only about 50% of the activity that was present at the onset of reproductive phase. On 28 DAA activity of GS equivalent to 412.5 and $456.5 \mu \text{ moles } \gamma\text{-GMH g}^{-1} \text{ h}^{-1}$ was still present in flag leaves of plants raised either normal or water deficit condition, respectively. Rate of decline in the activity was slow between 16 and 28 DAA. GS activity was affected by the environment only till 8 DAA after that enzyme activity in the samples collected from plants raised under irrigated as well as water deficit conditions was almost similar. Compared to NR and NiR activity of GS was least affected by the growing conditions.

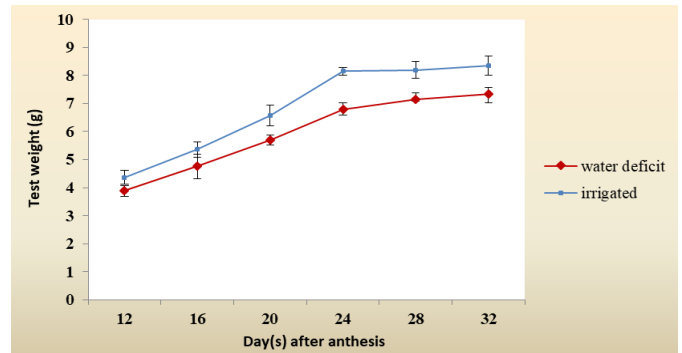


Fig 1: Test weight of developing grains of pearl millet hybrid HHB 94 grown under irrigated and water deficit condition

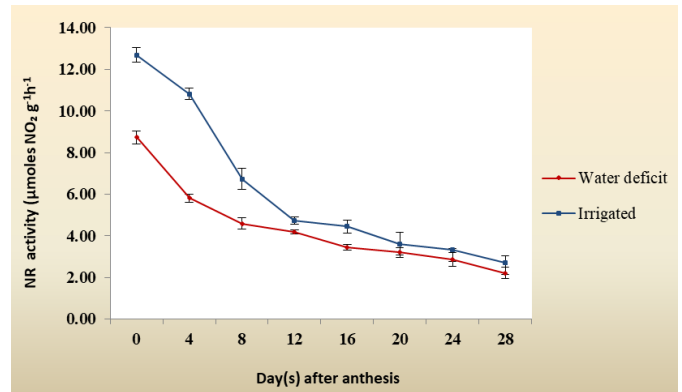


Fig 2: Activity of nitrate reductase (NR) in flag leaf of pearl millet hybrid HHB 94 grown under irrigated and water deficit condition

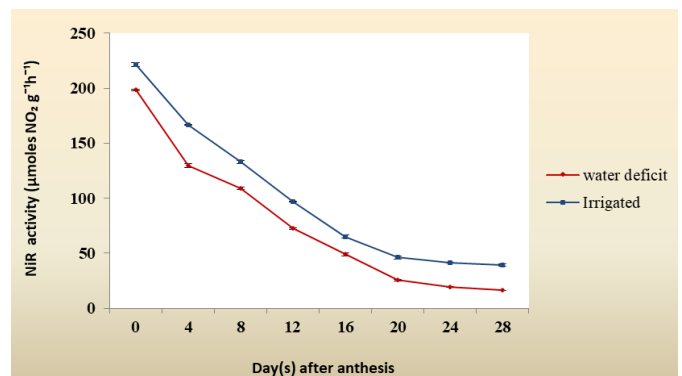


Fig 3: Activity of nitrite reductase (NiR) in flag leaf of pearl millet hybrid HHB 94 grown under irrigated and water deficit condition

Glutamate dehydrogenase (GDH)

Patterns of change in the activity of GDH in flag leaf of the pearl millet genotype HHB 94 grown under two different conditions are compared in Fig. 5. At the start of reproductive period *i.e.* on 0 DAA activity of GDH in plants grown under normal irrigated condition was $73.15 \mu \text{ moles NADH g}^{-1} \text{ h}^{-1}$ which increased slightly to 75.6 and $73.7 \mu \text{ moles NADH g}^{-1} \text{ h}^{-1}$ by 4 and 8 DAA. Thus the level of activity did not vary significantly till 8 DAA. Between 8 and 16 DAA the activity decreased by a margin of 20 % from 73.7 to $59.6 \mu \text{ moles NADH g}^{-1} \text{ h}^{-1}$ and thereafter no significant change in activity was observed till 28 DAA. Qualitatively a similar pattern of change in level of activity of the enzyme in leaves of plants grown under water deficit condition was observed, however,

the activity remained higher on all the days of sampling throughout the reproductive period of plant growth. For example activity of GDH in plants grown under water deficit condition on 8 and 16 DAA was 99.2 and 74.3 μ moles NADH $g^{-1} h^{-1}$, respectively whereas in plants grown under normal conditions for the same period was 73.7 and 59.6 μ moles NADH $g^{-1} h^{-1}$. The difference in the enzyme activities of water deficit and irrigated plants was even more at the start of reproductive period *i.e.* 0 DAA. In contrast to rapid decline in activity of NR and NiR, level of activity of GDH decreased slowly with increasing days after anthesis irrespective of the growing conditions. For example activity of NR in plants raised under normal irrigated condition declined by more than 60 % between 0 and 12 DAA (from 12.7 to 4.74 μ moles $NO_2 g^{-1} h^{-1}$) whereas activity of GDH, during the same period of growth declined only by 11% (from 73.1 to 65.1 μ moles NADH $g^{-1} h^{-1}$).

Aspartate aminotransferase (AspAT) and alanine aminotransferase (AlaAT)

Qualitative and quantitative pattern of change in the level of activity of aspartate aminotransferase in flag leaf of the genotype grown under normal irrigated and water deficit conditions is presented in Fig. 6. Throughout the reproductive period high enzyme activity was present in the leaves of irrigated plants. On the day of anthesis 5862 and 4884 μ moles NADH $g^{-1} h^{-1}$ of activity of AspAT was detected in plants grown under irrigated and water deficit condition, respectively. Similar to the other enzymes, activity of AspAT also decreased with increasing days of anthesis. Thus significantly lower activity was present in plants raised under water deficit condition. Though activity of this enzyme also declined with advancing grain formation but rate of decline was much slower compared to NR and NiR. For example activity of NR in plants raised under normal irrigated condition declined by more than 60 % between 0 and 12 DAA (*i.e.* from 12.68 to 4.74 $\mu g NO_2 g^{-1} h^{-1}$) whereas activity of AspAT, during the same period of growth, decreased only by 33 % (*i.e.* from 5862 to 3928 μ moles NADH $g^{-1} h^{-1}$). Similar to GDH a higher level of activity of AspAT was maintained during the period since flowering till physiological maturity of the grain under normal irrigated condition. Qualitatively pattern of change in activity of the enzyme in leaves of the plant grown under water deficit condition was similar to those grown under normal irrigated condition. It was also apparent that growing conditions had no significant effect on the activity. Activity of AlaAT was present only in traced in leaves of plants grown under normal as well as water deficit condition. No definite trend or pattern of change in activity of AlaAT in flag leaf of plants grown either normal or water deficit condition was observed.

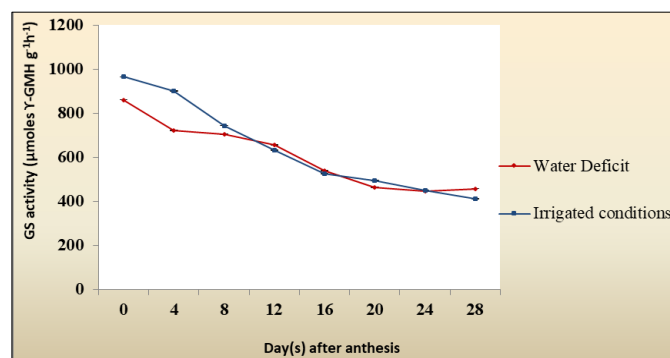


Fig 4: Activity of glutamine synthetase (GS) in the flag leaf of pearl millet hybrid HHB94 grown under irrigated and water deficit condition

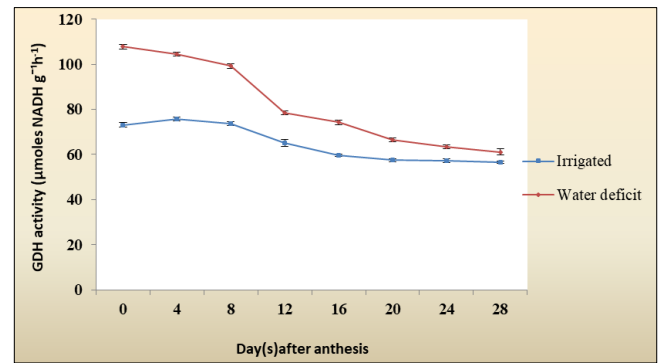


Fig 5: Activity of glutamate Dehydrogenase (GDH) in flag leaf of pearl millet hybrid HHB 94 grown under irrigated and water deficit condition

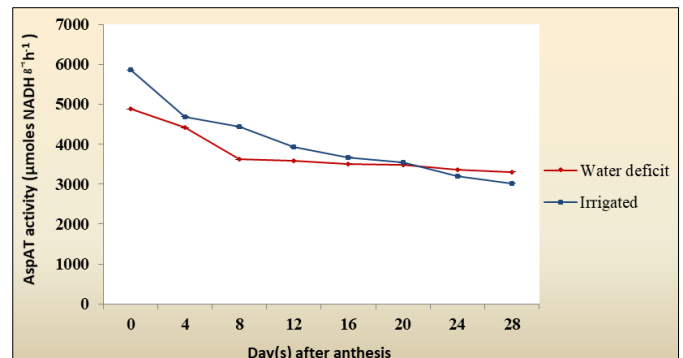


Fig 6: Activity of aspartate aminotransferase (AspAT) in flag leaf of pearl millet hybrid HHB 94 grown under irrigated and water deficit condition

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