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Impact of Flag Leaf Removal on Grain Development and Nutrients Deposition in Pearl Millet Developing Grains

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Abstract

Flag leaves play an important role in synthesis and translocation of photo-assimilates in the pearl millet plant, affecting grain yield; similarly it was believed to be a major source of remobilizing micronutrients (Fe and Zn) for the seeds. At the same time, the seed's sink strength plays an important role in dry matter accumulation. To investigate the relative contribution of pearl millet flag leaves to protein, micronutrient (Fe and Zn) and phytate accumulation, a field experiment was conducted to evaluate the effect of flag leaf removal on grain test weight (1000 grain weight), protein, micronutrients (Fe and Zn) and Phytate deposition in developing grains of two pearl millet cultivars *viz*. HC 20 and WHC 901-445. Flag leaf was removed on anthesis. Major effect of flag leaf removal was observed on test weight and protein content. A reduction of 10-12% in test weight and 4-6% in protein content was recorded in flag leaf removed plants than that of control plants. No significant reduction was observed in both the micronutrients (Fe and Zn), while phytate content behaved cultivar specific, a significant reduction was observed in HC 20 while it was unaffected in WHC 901-445. Perusal, a significant reduction was observed in all the nutrients in respect of test weight basis. From this investigation it is concluded that flag leaf plays vital role in grain maturation *i.e.* in supply of carbohydrate and protein fractions that might have limited role in micronutrient remobilization and transportation to developing grains in pearl millet.

Keywords

Pearl millet; Phytate; Protein; Developing grains

Abbreviations: Wt: Weight; DAA: Days after anthesis; FLR: Flag leaf removed; G: Gram; Vs: Verses.

Introduction

In cereals, grain yield is mainly dependent on photosynthetic source-sink relationship dictating changes in carbohydrate synthesis, accumulation and partitioning. Flag leaves play a major role in synthesis and translocation of photo-assimilates to the cereal seeds, affecting grain yield. The top two leaves are considered the primary source while the developing grains are the primary sink [1]. In rice, the top three leaves including the flag leaf export assimilates to the panicle [2]. Mae [3] reported that 60–90% of total carbon in the panicles at harvest is derived from photosynthesis after heading, while 80% or more of nitrogen (N) in the panicles at harvest is absorbed before heading and remobilized from vegetative organs. Abou-Khalifa et al. [4] reported that flag leaf contributed to 45% of rice grain yield and, when removed, was the major component for yield loss. In wheat, defoliation of the flag leaf blade increased the contribution of assimilates to grain from the stem and the chaff under normal conditions [5]. Overall, removal of flag leaf adversely affected grain yield under normal or water limiting conditions in cereals such as rice [6-9], wheat [10,11] and barley [12]. In wheat, up to 34.5% grain yield reduction was reported after flag leaf removal at the heading stage [13], while Birsin [14] showed that flag leaf removal resulted in 13, 34 and 24% reduction in grain per spike, grain weight per spike and 1000-grain weight, respectively, and 2.8% increase in grain protein contents. Leaf senescence during reproductive and ripening stages is directly related to biomass production and grain yield of rice crop [15-17]. The top three leaves not only assimilate majority of carbon for grain filling during ripening phase, but provide large proportion of remobilized-nitrogen for grain development during their senescence [3]. Similarly, rice flag leaves are also believed to be a major source of remobilized minerals for the seeds, and recent reports tried to correlate gene expression levels on flag leaves with concentration of mineral nutrients in rice seeds [18,19]. Sperotto et al. [20] reported that flag leaf is not necessary for metal remobilization to the seeds and that of seed sink strength. However, in pearl millet, no single report is there on this aspect as well as not a single report has pointed role of flag leaf in phytate deposition to the developing seeds in any crop. Keeping these views in mind the present investigation was carried out to investigate the relative contribution of pearl millet flag leaves to protein, micronutrient (Fe and Zn) and phytate accumulation in developing grains.

Material and Method

Two pearl millet cultivars (HC 20 and WHC 901-445) were grown at CCS Haryana Agricultural University, Hisar research Farm (Genetics and Plant Breeding area) with recommended agronomic package and practices during kharif-2014. Almost 100 similar looking plants from each cultivar were tagged before anthesis and just after anthesis flag leaf was removed from 50 plants of each cultivar. The ear head of 10 plants along with 10 control plants from each cultivar were collected at 16, 24 and 32 days after anthesis. Dried them at room temperature and grains were collected separately. These grains were used for investigation and each investigation was carried out in three replications.

For estimation of 1000 grain weight, grains were counted manually as well with the help of seed counter and weighed them on weighing balance (Mettler Toledo, ML204/Ao1). Total Protein in grains was estimated by Micro-Kjeldahl method [21]. Phytic acid was determined by employing the method of Haug and Lantgsch. Finely ground sample (500 mg) was extracted with 25 ml of 0.2 N HCl for 3 hours with continuous shaking on orbital shaker. After proper shaking it was filtered through Whatman No. 1 filter paper. The filtrate was used for Phytate estimation. An aliquot (0.5) of above sample extract was taken in a test tubes and 0.9 ml distilled water was added. To all the tubes 1 ml 0.02% ferric ammonium sulphate solution (prepared in 0.2N HCl) was added and then placed in a boiling water bath for 30 minute. One ml of supernatant was transferred to another test tube and 1.5 ml 1% bipyridine solution was added. The absorbance was measured UV–Vis spectrophotometer (Thermo Scientific, Evolution

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201) at 519 nm against distilled water blank. Phytate was calculated by using standard curve of sodium Phytate (200 µg/ml). Fe and Zn were estimated by Energy Dispersive X-rays fluorescence (EDXRF), at ICRISAT Patancheru, Hyderabad, method described by [22].

Results

To evaluate the relative importance of flag leaves as sources of photosynthates, nitrogen, remobilized micronutrients (Fe and Zn) and Phytate to pearl millet grains, we analyzed the effects of flag leaf removal. Under this experiment two pearl millet genotypes were included i.e. HC 20 and WHC 901-445 and flag leaf was removed just after anthesis. Randomly ten ear heads each from flag leaf removed and control plants were collected at 16DAA, 24DAA and 32DAA, dried them at room temperature and analyzed for their protein, phytate, iron and zinc content along with control plant. The mean values of test weight, protein, phytate, Fe and Zn contents as well as their deposition (test weight basis) are given in tables. A Significant reduction (10-12%) in test weight was recorded at every grain developmental stage irrespective of the composite. Test weight recorded in control vs. flag leaf removed plants at different grain maturity stages was 7.11 vs. 6.19, 9.75 vs. 8.92 and 11.28 vs. 10.37g in HC 20 while 7.86 vs. 7.76, 11.96 vs. 9.71 and 12.89 vs. 11.23g in WHC 901-445 respectively, at 16, 24 and 32DAA (Table 1 and Figure 1). In HC 20 higher reduction in test weight was observed at early grain developmental stage (16DAA) compared to harvest mature grains, while in WHC 901-445 reverse trend was observed.

Like test weight, protein content was gradually decreased in both the cultivars but to a lower extent (4 - 6%) at harvest mature grains in flag leaf removed plants as compared to control plants. The protein content recorded in control *vs.* flag leaf removed plants at different grain maturity stages was 12.03 *vs.* 9.43, 11.74 *vs.* 10.88 and 11.94 *vs.* 11.44% in HC 20 while 14.15 *vs.* 10.68, 13.76 *vs.* 11.86 and 13.44 *vs.* 12.65% in WHC 901-445 genotype (Table 1). The reduction in protein content was observed at all the grain developmental stages (Figure 2) but a drastic reduction in protein content was observed at early grain maturity stage (16 DAA) i.e. 21.61% in HC 20 and 24.52% in WHC 901-445, but it recovered up to 7.33% and 13.81% level at 24 DAA and to 4.27 and 5.88% in harvest mature grains at 32 DAA, in HC 20 and WHC 901-445, respectively. In respect of per grain basis (Test weight), the reduction in protein content was recorded at higher extent i.e. 13.45 and 18% at harvest mature grains in HC 20 and WHC 901-445, respectively.

Unlike the test weight and protein content, no significant variation was observed in micronutrients (Fe and Zn) content in grains recovered from control vs. flag leaf removed plants at any grain filling stage irrespective of the composites (Table 2). Perusal, the data presented in table showed a significant reduction from 13 to 16% in

Table 1: Impact of Flag leaf removal on Test weight and Protein deposition in developing grains of pearl millet. *FLR- Flag leaf removed, wt-weight, DAA- Days after anthesis.

Growth stages	Test Weight (g/1000 grains)		% Reduction in test wt.	Protein (g/100 g)		% Reduction in protein	Protein (g/test wt.)		% Reduction in protein
	Control	FLR		Control	FLR		Control	FLR	
HC 20									
16 DAA	7.11	6.19	12.9	12.03	9.43	21.61	0.86	0.58	31.76 15.21461 13.44528
24 DAA	9.75	8.92	8.5	11.74	10.88	7.33	1.14	0.97	15.22
32 DAA	11.37	10.28	9.6	11.95	11.44	4.27	1.36	1.18	13.45
WHC 901-445									
16 DAA	7.86	7.26	7.6	14.15	10.68	24.52	1.11	0.78	30.28
24 DAA	11.96	10.71	10.45	13.76	11.86	13.81	1.65	1.27	22.82
32 DAA	12.89	11.23	12.9	13.44	12.65	5.88	1.73	1.42	18.00
CD				0.46	0.40				

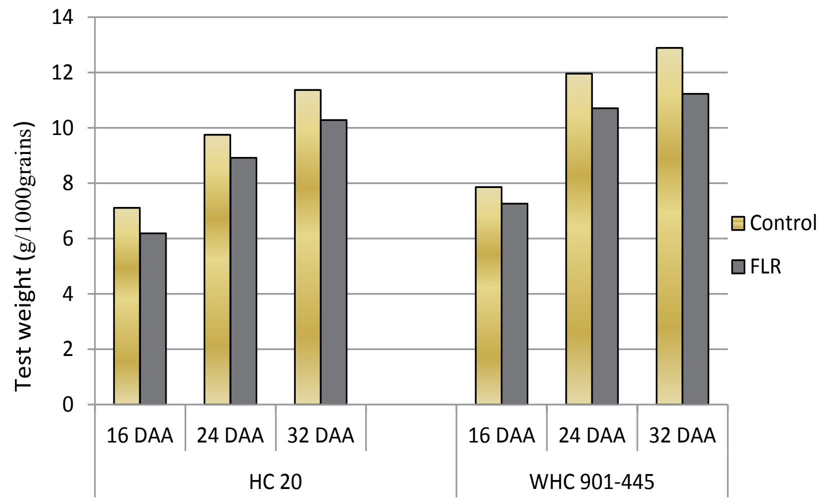

Figure 1: Impact of Flag leaf removal on Test weight in developing grains of pearl millet.

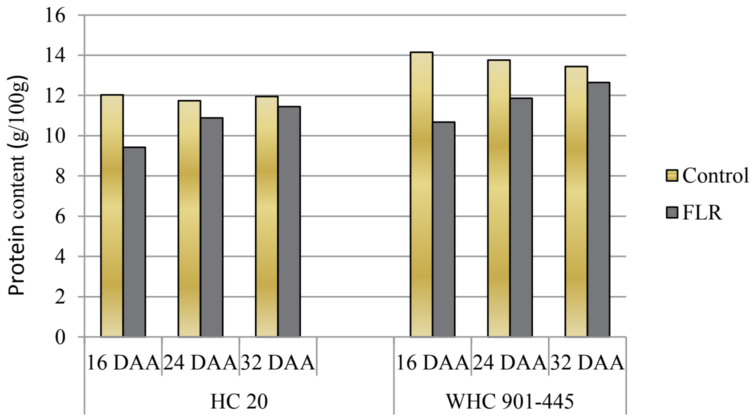

Figure 2: Impact of Flag leaf removal on Protein deposition in developing grains of pearl millet. *FLR- Flag leaf removed, DAA- Days after anthesis.

Table 2: Impact of Flag leaf removal on micronutrient (Fe and Zn) deposition in developing grains of pearl millet. *FLR- Flag leaf removed, wt-weight, DAA- Days after anthesis.

Growth stages	Fe (mg/kg)		Fe (mg/test wt)		% Reduction in Fe (test wt basis)	Zn (mg/kg)		Zn (mg/test wt)		% Reduction in Zn (test wt basis
	Control	FLR	Control	FLR		Control	FLR	Control	FLR	
HC 20										
16 DAA	78	76	0.555	0.470	15.17	39	44	0.277	.272	1.78
24 DAA	69	70	0.673	0.624	7.19	39	41	0.380	0.366	3.82
32 DAA	72	69	0.819	0.709	13.35	45	43	0.512	0.442	13.61
WHC 901-445										
16 DAA	74	65	0.582	0.472	18.87	55	55	0.432	0.399	7.63
24 DAA	77	76	0.921	0.814	11.61	57	53	0.682	0.568	16.74
32 DAA	76	73	0.980	0.820	16.32	56	57	0.722	0.640	11.32
CD	3.86	3.60				3.04	3.14			

Table 3: Impact of Flag leaf removal on phytate deposition in developing grains of pearl millet. *FLR- Flag leaf removed, wt-weight, DAA- Days after anthesis.

Growth stages	Phytate (mg/g)		% Reduction in Phytate	Phytate (mg/test wt)		% Reduction in Phytate (test wt basis)
	Control	FLR		Control	FLR	
HC 20						
16 DAA	6.30	5.53	12.22	0.045	0.034	23.58
24 DAA	6.30	5.23	16.98	0.061	0.047	24.05
32 DAA	6.50	5.44	16.31	0.074	0.056	24.33
WHC 901-445						
16 DAA	6.79	6.70	1.33	0.053	0.049	8.86
24 DAA	6.60	6.42	2.73	0.079	0.069	12.89
32 DAA	6.60	6.74	-2.12	0.085	0.076	11.03
CD	0.15	0.18				

iron and 11 to 13% in Zn content in respect of test weight basis at harvest mature grains. On 32 DAA, HC 20 contained 0.798 vs. 0.699 mg/test weight Fe and 0.508 vs. 0.446 mg/test weight Zn while WHC 901-445 contained 0.978 vs. 0.819 mg/test weight Fe and 0.719 vs. 0.640 mg/test weight Zn in grains produced by control *vs.* flag leaf removed plants, respectively.

Perusal, the data presented in Table 3 showed that both the composites (HC 20 and WHC 901-445) behaved differently in respect of flag leaf removal effect on phytate content. A significant reduction (16.31%) was recorded in phytate content in the grains produced by flag leaf removed plants of HC 20, while no significant effect was observed in if phytate content of grains produced by control *vs.* flag leaf removed plants of WHC 901-445 (Table 3). In respect of per grain basis phytate deposition, a significant reduction in phytate deposition (24.33% in HC 20 and 11.03% in WHC 901-445) was recorded. It is interesting to note that grains that are produced by flag leaf removed plants of both the composites showed a slight reduction in phytate content at 24 DAA than that of 16DAA and then again recovered to its almost initial level at 32 DAA. It was observed that it decreased from 5.53 (16 DAA) to 5.23 mg/g (24 DAA) and recovered to 5.44 mg/g (32 DAA) in HC 20 and from 6.70 (16 DAA) to 6.42 mg/g (24 DAA) and recovered to 6.74 mg/g (32 DAA) in WHC 901-445.

Discussion

In cereals, grain yield is mainly dependent on photosynthetic source-sink relationship dictating changes in carbohydrate synthesis, accumulation and partitioning. The top two leaves are considered the primary source while the developing grains are the primary sink [1]. In the present investigation, on flag leaf removal a drastic reduction (10-12%) in grain test weight (1000 grain weight) was recorded in

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pearl millet. No such reports are available in pearl millet or other millets. But similar results were also reported by earlier researched in wheat, barley and rice. Overall, removal of flag leaf adversely affected grain yield under normal or water limiting conditions in cereals such as rice [6-9], wheat [10,11] and barley [12]. In wheat, up to 34.5% grain yield reduction was reported after flag leaf removal at the heading stage [13], while Birsin [14] reported a reduction of approximately 34 and 24% in grain weight per spike and 1000-grain weight respectively on flag leaf removal. In the present investigation, like test weight, protein content was also decreased in both the cultivars but to a lower extent (4 - 6%) at harvest mature grains in flag leaf removed plants as compared to control plants. But these results were not matching the previous reports in other cereals like wheat. Birsin [14], reported 2.8% increase in grain protein content of flag leaf removed plants than that of control plant in wheat. It is reported in literature that top three leaves not only assimilate majority of carbon for grain filling during ripening phase, but provide large proportion of remobilized-nitrogen for grain development during their senescence [3,16]. On flag leaf removal a sudden drastic reduction in protein content at early grain filling stage (16 DAA) is recovered at later grain filling stages might be due to make shift arrangements made by plant to supply nitrogen/protein to the developing grains from other plant part like stem and lower leaves in the absence of flag leaf. Similarly, rice flag leaves are also believed to be a major source of remobilized minerals for the seeds, and recent reports tried to correlate gene expression levels on flag leaves with concentration of mineral nutrients in rice seeds [18,19]. No such reports are available in literature in millets as well as these type of information are scanty in other cereals also. The research findings of this present investigation showed that there are no significant effect of flag leaf removal on micronutrient deposition in developing grains of pearl millet. These results are completely matching with the earlier reports in other cereals like rice. Sperotto et al. [19] did not observed any effect of flag leaf removal on Fe and Zn content in rice grains and concluded that flag leaf is not necessary for metal remobilization to the seeds and that of seed sink strength. These authors removed the second upper leaf and found similar results. Several other studies also emphasized the importance of remobilization of reserves to supply rice seeds with minerals [23-25], but the factual role of stored minerals to total seed micronutrient content is uncertain. Our data show that remobilization from flag leaves in pearl millet is not absolutely required for seeds to acquire minerals in plants growing in field conditions. There is a possibility that flag leaves might be preferential but not essential as a source of micronutrients (Fe and Zn) and flag leaf removal can probably be compensated by other plant parts *viz.* other leaves and stem/sheath remobilization and/or continuous uptake by roots. Leaf Senescence in cereals is regulated at the individual leaf level by mobilizing nutrients from older leaves to younger leaves and finally to the flag leaf [26,27]. Therefore, it is possible that in the absence of flag leaves, as during senescence a plant without flag leaves could remobilize nutrient pools from other sources directly to the grains and would yield similar Fe and Zn concentrations. As the results showed that there is genotypic dependent impact of flag leaf removal on phytate deposition in developing grains in pearl millet. But at the earlier grain developmental stage a slight reduction was observed during the phytate deposition. It indicates that flag leaf removal reduces the phytate deposition in developing grains but at maturity stage it might be synthesized and/or transported to the developing grains from the other plant parts like stems/sheath or other leaves. No such reports are available in the about phytate deposition in developing grains as well as the effect of flag leaf removal on

Conclusion

From this investigation it has been concluded that flag leaf plays an important role in synthesis and translocation of photo-assimilates and nitrogen supply for storage protein synthesis to the pearl millet seeds, affecting grain yield (test weight) and protein content, while it might have very low significance in respect of Fe and Zn contents of pearl millet grains while for making any conclusions on effect of flag leaf on phytate content of pearl millet grains, a fair number of lines/genotypes to be studied.

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