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Original article

Evaluation of protein, monosaccharide, free proline content during grain filling in *Triticum Aestivum V. Zarrin*

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ABSTRACT

The progress of seed development was accompanied change in pattern of biochemical and physiological parameter which affected by environmental condition and developmental stage during grain filling period. We performed several studies to understand the biochemical and physiological events taking place during seed grain filling. In this investigation, we studied changes in protein, monosaccharide and free proline content as biochemical parameter and fresh weight as a growth parameter during grain filling (8weeks) period at field growing Iranian wheat cultivar (Triticum aestivum v. zarrin) . Results indicate that the most increase in fresh weight occurs between first and second week after anthesis. Constant phase of weight change occurred in 2, 3 and 4 week age. Significant decrease in fresh weight occurs in 5 week age of grain filling. Increase in protein content at 6 week age of grain may be related to biosynthesis of late-embryogenesis-abundant (LEA) proteins that thought to be involved in desiccation during dehydration phase of seed development in wheat grain not similar to stress condition.

1. Introduction

Cereal grains (wheat, rice, maize, barely, etc) constitute the world's primary crops and the cereal endosperm provides the major source of carbohydrates and proteins for human food and livestock feed (Black, et al., 1999) .The processes involved in wheat grain development are yet poorly understood despite the importance of cereals as a major source of nutrition for human kind. In monocotyledonous plants, the process of seed development involves the deposition of reserves in the starchy endosperm and development of the embryo and aleurone layer. The final stages of seed development accompanied with an increase in desiccation tolerance and drying out of mature seed (Finnie, et al., 2002). Mature wheat grains cantain 8-20% protein. The abundant gluten proteins constitute up to 80% of total flour protein(Cornell, et al., 1998). In mature grain the storage proteins, gliadins and glutenins are aggregated in polymers with different sizes and solubility. Distribution of monomeric and polymeric proteins as well as their solubility plays a critical role in governing wheat flour properties, including baking quality (Dupont,et al., 2003). Most emphasize the role of hormones in the embryo or aleurone during seed development and germination. Many other compounds produced in roots, leaves and seeds are candidates for regulatory compounds that influence grain fill. The supply of photosynthute is a major factor that limits accumulation of dry weight (Dupont,et al., 2003). Sugars and amino acids from the leaves and stems not only serve us substrates for starch and protein synthesis but also interact to regulate gene expression and metabolic pathways. Sugars act as growth regulating in seeds (Dupont,et al., 2003). The balance between nitrogen, sulphore and carbon compound may be involved in regulation of amino acid biosynthesis, catabolism and accumulation of proteins. Plant and kernel water status, hormone status as well as physical processes play major role during grain fill (Dupont,et a.,l 2003). Compatible solutes known as compatible osmolytes, they are small group of chemically diverse organic compounds that are highly soluble and do not interfere with cellular metabolism, even at high concentrations (Buchanan, et a.,l 2000, Sanchez, 2000). Synthesis and accumulation of organic osmolytes are widespread in plants, but the distribution of specific compatible solutes varies among plant species. The amino acid proline is accumulated by taxonomically divers set of plants. One mechanism for increasing solute concentrations is the irreversible Synthesis of compounds such as glycine betaine. Concentrations of other compatible solutes (e.g. proline) are maintained through a combination of synthesis and catabolism. Monomeric sugars (e.g. glucose and fructose) can be released from polymeric forms (starch, fructans) in response to stress (Buchanan, et al., 2000). Despite of the larg number of studies concerning the effect of environment and cultivar – environment interaction on the variation of biochemical compound there is a few data concerning the time course accumulation. The aim of present work was to understand, quantify and compare changes in some biochemical compound concentration and accumulation, during wheat grain filling. This investigation seeks to further clarify the time effects on grain quality parameter of wheat using filed grown condition, in relation to grain filling.

2. Materials and methods

1.2. Plant material

Triticum aestium (zarrin) was field grown in Sahibabad, Urmia, Iran, under supervision of urmia agriculture research center. The beginning time of wheat cultivation in the field has been at October (2004). Seeds were collected at weekly intervals over a period of 8 week during the grain filling and maturation stage at the first week until one week after harvesting. Our experiments, carrid out in conditions close to the Mediterranean climate. For statistical differences decreasing between samples, all samples were in same growing condition and collected in one homogen bloke with 1*1 diameters also in each sample seed collected only from middle of glum. The collection time and data was shown in Table 1. At first fresh weight of samples was measured. For other chemical analyses samples stored in -4º C until need.

2.2. Protein content

Protein content of samples was determined by Folin-lowry method (Bollage, et al., 1990). This method providing greater sensitivity, so that quantities of protein less than 1 mg could be assayed accurately (Lowry, et al., 1950).

2.3. Proline content

Proline content determined using the Bates method (Bates, 1973). All proline, protein, and soluble monosaccharide assays were based on drought weight of grains (Waldren,et al., 1974).

4.2. Soluble monosaccharide concentration

Phenol-sulphoric acid assay (Chaplin, et al., 1987), (Dubois, et al., 1951) used for measurement of monosaccharide concentration change during grain filling. It is useful as a rapid non specific method. This assay have been developed which rely on the action of concentrated sulphoric acid causing hydrolysis of all glycosidic linkage and subsequent dehydration of the monoccharides released to give derivative of furfural. The dehydration products react with a number of compounds such as phenol to give colored products. Whilst total sugar concentrations are readily obtained for homoglycans, care must be taken in interpreting the results obtained with heteroglycans due to the different couloirs intensities produced by different monosaccharide (Chaplin, 1987).

3. Results and discussions

The progress of wheat seed development was accompanied by change in pattern of biochemical and physiological parameter which affected by environmental condition and developmental stage during grain filling period (Daniel, et al., 2002). Grain development could be dividing in to, early, medium and late milk stages, and early, soft and hard dough stages (Gebbing, et al ., 1999). The temporal pattern of grain development also can be described in terms of transition pointes in accumulation of total dry mater, starch, protein and water (Gooding, et al., 2003).

Table 1
Changes in protein, monosaccharide, free prolin content and fresh weight changes during grain filling.

	Fresh weight (g) Mean± Std	Protein(mg/g.li) Mean± Std	Monosaccharide(mg/g.li) Mean± Std	Prolin (µM/g) Mean± Std
1	0.032233±.0012342	3.997680±.1160677	2.6333±.10214	85.465400±27.2710152
2	0.064600±.0032512	3.976800±.2101682	0.9371±.16180	188.359633±9.7150996
3	0.066000±.0024434	2.853400±.0975814	0.9540±.00291	357.841200±32.3077154
4	0.063633±.0007095	6.349767±.2655200	0.8502±.02342	291.520900±30.9432635
5	0.044967±.0006110	6.696033±.0179305	0.2335±.01469	398.254033±12.1278793
6	0.045300±.0025942	17.690667±.2279040	0.1936±.00737	255.603567±27.7684745
7	0.046633±.0023438	13.706267±.0216472	0.2297±.00737	285.615767±6.0184092
8	0.045233±.0008505	10.650967±.4516529	0.3249±.01913	249.180167±36.2629600
Total	0.051075±.0117816	8.240197±5.0615595	0.7945±.78070	263.980083±95.1065710

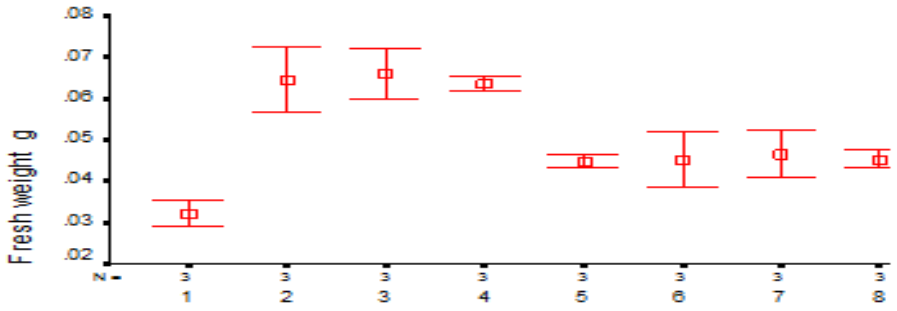

Fig. 1. Change in fresh weight of wheat kernel during 8 weeks of grain filling.

In order to pin point time in grain development when changes in biochemical and physiological parameter are likely to occur, age of kernel (weekly) considered as a factor and 4 biochemical and physiological parameters considered as a variable in completely randomized design and analyzed with the statistical software program SPSS. Data subjected to analysis of variance (ANOVA) procedures. In this study we evaluate fresh weight, protein content, soluble monosaccharide sugars and free proline concentration in one complete kernel of field-grown wheat (Triticum aestivum var zarrin) during 8-week post seed formation. Figure 1, Table.1 shows fresh weight changes during 8 weeks of grain filling.

The most increase in fresh weight occurs between first and second week after seed formation. Constant phase of weight change occurred in 2, 3 and 4 week age. Significant decrease in fresh weight shown in 5 week age of grain fills. Table 3 shows Analysis of variance for the effect of age on Wet weight changes during grain filling. Table 3 shows Means comparison of wet weight changes during grain filling with Duncan test.

Figure 2 shows protein content changes in 8 groups of samples (weekly collected). Table2 shows Protein content changes during grain filling. ANOVA Table 3 and Duncan test Table 3 shows significant difference (sig .000) between groups. Seeds with 6week age have highest protein content and the must accumulation of protein occure in this time.

Table2
Analysis of variance for the effect of age on biochemical parameters.

variables	Sum of Squares	df	Mean Square	F	Sig.
Fresh weight	0.003	7	0.000	112.138	0.000
Protein content	588.457	7	84.065	1705.239	0.000
monosaccharide	13.937	7	1.991	390.957	0.000
	197823.45	7			0.000

Prolin	28260.493	44.254

Protein synthesis also affected by dehydration phase. Increase in protein content at 6 week age of grain may be related to biosynthesis of late-embryogenesis-abundant (LEA) proteins that thought to be involved in desiccation tolerance. Two other groups of proteins the RAB (responsive to Abscisic acid) and DHN (dehydrin) also promote by ABA during the mid-to late stages of seed development, they act specifically in the protection of membranes and other proteins against desiccation damage (Taiz, et al., 1998).

Figure 3 and Table1 shows changes in soluble monosaccharide concentration during grain filling. Table 2 shows Analysis of variance for the effect of age on Monosaccharide content changes. Monosaccharide concentration determined based on phenol-sulphoric acid assay, homoglycans total carbohydrate concentration obtained by the assay. While starch accumulation occur in endosperm during grain filling, decrease in soluble monosaccharide concentration, which consider as a monomer units for starch was shown by our results. In one week age seeds we see high concentration of soluble sugars, but in mature seeds when starch accumulation is completed, minimum content of soluble Monosaccharide is shown. Soluble monosaccharide content as an osmoregulater-increased response to stress, also our result shows that monosaccharide content of grain decrease during dehydration phase. Figure 4 and Table2 shows change in free proline concentration at different Stage of grain filling. ANOVA Table3 show significant differences between groups. Duncan test Table 4 homogenized groups in four subset. Increase of Proline content in second week compare to first week is significant, but difference between 3, 4,5and6 week age grains and between 7 and 8 week is non-significant. Free proline and soluble sugars considers as osmoregulaters. By increasing osmotic stress (drought, saline, etc)proline content and soluble sugars in leaves and roots of seedling significantly increased .Recent studies shown that the free proline content of excised leaves of many important agriculture crops increase markedly with drought stress .our result demonstrated that Free proline and soluble sugars content change during dehydration phase of seed development in wheat grain not similar to stress condition. Figure 4 indicate that despite of decrease in water content of seed during dehydration phase in seed development after fourth week proline content did not show significant increase .therefore proline content changes of wheat grains during dehydration phase of grain filling not same as a stress condition. Seed development can be divided in to two phase of approximately equal duration (Sotres, et al., 2000). During the first phase cell divisions, embryogenesis and endosperm tissue proliferates occure. During the second phase cessation of cell divisions, dehydration and development arrest. During the second phase, storage compounds accumulate. The embryo become tolerant to desiccation and the seed dehydrates, losing up to 90% of its water (Taiz, et al., 1998).

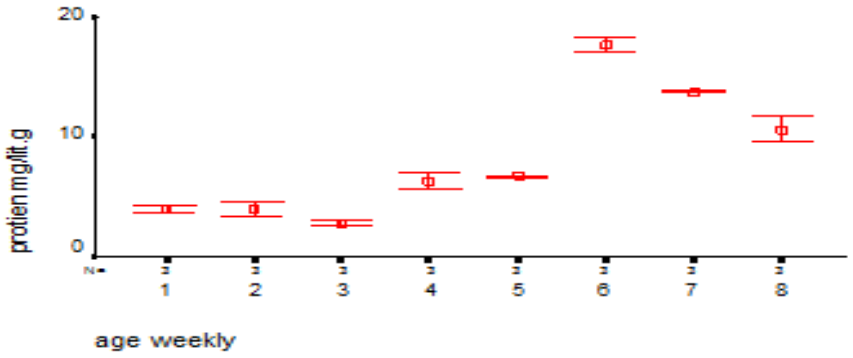

Fig. 2. Protein content change in wheat kernel during 8 weeks of grain filing.

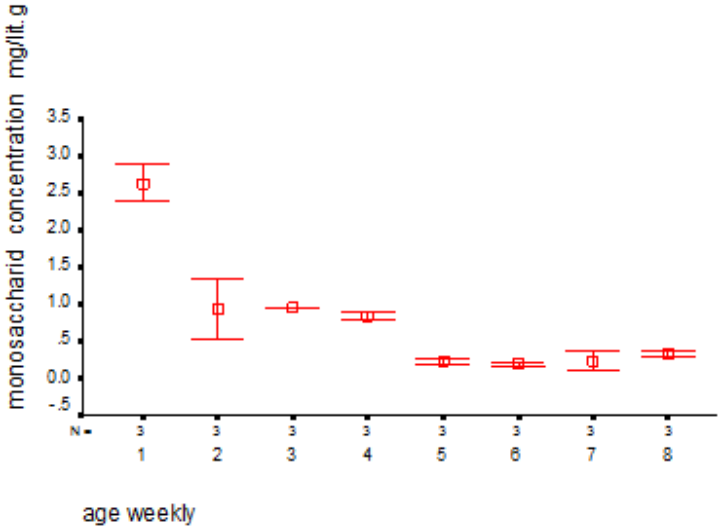

Fig. 3. Soluble monosaccharide concentration change in wheat kernel during 8 weeks of grain filing.

Since seeds of different developing stage exist on plants even as late as 8-9Weeks after the beginning of flowering, it is very difficult to predict the optimal harvest time. Analysis such as our study can help seed producer to fix the most economic harvest data. Sugars are reputed to protect membrance in dehydrated desiccation – tolorant organisms, such as seeds and pollen. They interact with the polar head groups of the membrane phospholipids; because the amount of sugars may be in sufficient for full interaction in some organism another mechanism of membrane protection was sought (Hoekstra, et al., 2000, Haidari, et al 2003, Ross, et al., 1974).

This investigation seeks to further clarify the time effects on grain quality parameter of wheat using filed grown condition, in relation to grain filling. Results indicates that despite of dehydration phase in seed development, changes in the parameters did not showed stress condition, also as a consequence of dehydration metabolism comes to a quiescent state. Biosynthesis of late-embryogenesis-abundant(LEA) proteins and two other groups of proteins the RAB (responsive to Abscisic acid) and DHN (dehydrin) that also promote by ABA during the mid-to late stages of seed development, these proteins thought to be involved in desiccation tolerance

and they act specifically in the protection of membranes and other proteins against desiccation damage. These Protein protection mechanisms led to that despite of 90%decrease in water content during dehydration phase of grain filling the stress parameter that we studied did not increased significantly.

Table 3

Means Table 3. Means comparison of biochemical content changes during grain filling with Duncan test.

Age weekly	Fresh weight	protein content	Prolin content	Monosaccharide content
1	.032233 [a]	3.997680 [b]	85.465400 [a]	2.6333 [c]
2	.064600 [c]	3.976800 [b]	188.359633 [b]	.9371 [b]
3	.066000 [c]	2.853400 [a]	357.841200 [d]	.9540 [b]
4	.063633 [c]	6.349767 [c]	291.520900 [c]	.8502 [b]
5	.044967 [b]	6.696033 [c]	398.254033 [d]	.2335 [a]
6	.045300 [b]	17.690667 [f]	255.603567 [c]	.1936 [a]
7	.046633 [b]	13.706267 [e]	285.615767 [c]	.2297 [a]
8	.045233 [b]	10.650967 [d]	249.180167 [c]	.3249 [a]

Subset for alpha = .05 Means for groups in homogeneous subsets are displayed. A Uses Harmonic Mean Sample Size = 3.000.

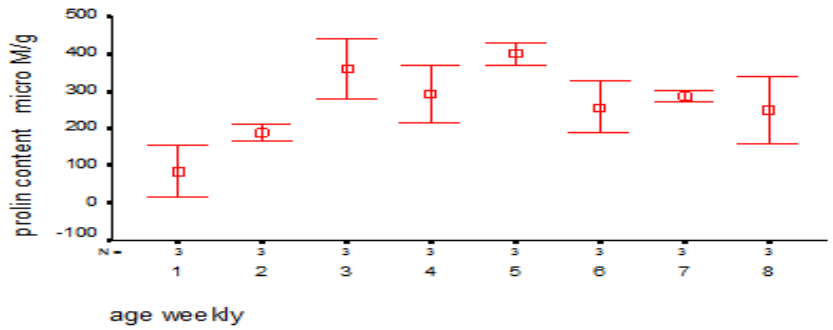

Fig. 4. Free prolin content change in wheat kernel during 8 weeks of grain filing.

4. Conclusion

Results indicate that despite of dehydration phase in seed development, changes in these parameters did not show stress condition, although metabolism comes to a quiescent state because of dehydration. Our result show

biosynthesis of late-embryogenesis-abundant proteins, and dehydrated proteins the responsive to abscisic acid during the mid-to late stages of seed development, these proteins are responsible for reduction in stress damage during dehydration phase in seed development.

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