INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 1, p. 339-345, 2019

RESEARCH PAPER OPEN ACCESS

Polyphenol oxidase (PPO) activity during germination and early seedling growth of wheat (*Triticum aestivum*) and effect of seed size, abrasion and light on its activity

Neelam Taj*, Syed Zahir Shah

Department of Botany, Islamia College University, Peshawar KP, Pakistan

Key words: Polyphenol oxidase (PPO), Germination, Abrasion, Bran.

http://dx.doi.org/10.12692/ijb/14.1.339-345 Article published on January 26, 20199

Abstract

The most important widespread cereal is Wheat (*Triticum* spp) which is used throughout the world. An important problem for the flour and flour related products is their darkening and discoloration which is believed to result from polyphenol oxidase (PPO) activities. The most important enzyme responsible for browning of end product in wheat is Polyphenol oxidase (PPO). The factors affecting PPO activity may be germination, light; seed size during germination of wheat grains, the Polyphenol oxidase activity was studied in wheat grain. Increase in PPO activity was observed in bran tissues at the start of germination and gradual decrease with time on 4th day of germination occurred. An increase in PPO activity was observed up to 4th day and it was decreased on the 5th, 6th, and 7th day of germination in both embryo and endosperm, while increased in young seedlings. The effect of light on Polyphenol oxidase (PPO) activity in wheat grain was monitored during germination increased in (PPO) activity was recorded. A successive decrease in seed weight and rising in abrasion time was documented. Stability of PPO is clear in dry seeds and the minor harm come across during harvest or threshing which does not make significant rise and decline in enzymatic action. High enzymatic action was noted in large seed than the smaller ones. A successive decrease in seed weight and rising in abrasion time was documented. Stability of PPO is clear in dry seeds and the minor harm come across during harvest or threshing which does not make significant rise and decline in enzymatic action. Main emphasis of this study was on evaluating PPO activities during germination and factors affecting its activity.

* **Corresponding Author:** Neelam Taj ✉ Neelamtaj2013@gmail.com

Introduction

PPO is an enzyme containing copper involved in the catalysing of two different reactions: i.e (1) hydroxylation in which monophenol is converted to O-diphenols (monophenolmonooxygenase: E.C. 1.14.18.1) and (2) oxidation in which odiphenolscovertion to o-quinones (diphenol oxidase: E.C. 1.10.3.2) occurs. Natural polymerization of O-Quinone to melanins will occur, the dark color pigment with high molecular weight (Zaini *et al.,* 2013)). Enzymatic browning of cereal products, vegetables and fruits are in association with Polyphenol oxidases (PPOs) enzymatically. Dark colour of noodles and various products of wheat that are dependent on time have been investigated for PPOs and it was found that PPO is the main agent responsible for it (Feillet *et al.*, 2000).

Grown all over the world wheat covers more of the earth's surface than any other cereal crop (Kayani *et al.,* 2010). Wheat is the major food for the people of Pakistan and is used in the form of chapatti, bread, porridge and many bakery items.

In industry of vegetables and fruits, Enzymatic browning is of major importance. This kind of browning causes reduction in shelf life of many processed foods causing discoloration, and also causes effects on the production of vegetables and fruits that are frozen and dehydrated Even though discoloration caused by enzymatic browning occurs but on the other hand in processes like tea and cocoa fermentation, and also in the formation of products of prune, date and raisin, it is a required reaction.

The enzymes as tyrosinases or PPO are having dinuclear copper center, that are capable for inserting oxygen into an aromatic ring, in a pose as ortho- to already present hydroxyl group. After this oxidation of the diphenol to the corresponding quinone occurs and therefore molecular oxygen is set free in the reaction.

Active site of enzyme, having extremely preserved structure having copper bounded by a single cysteine residue and six or seven histidine residues. This enzyme in, animals, plants fungi and bacteria has universal distribution. There is a lot of ignorance regarding biological function of it, particularly in plants and also in fungi (Mayer, 2006).

Materials and methods

Grains of 10 different under study wheat cultivars were procured from Nuclear Institute of Food and Agriculture (NIFA) Tarnab KP, for their polyphenol oxidase study during germination.

Incipient germination

Grains of all of the ten cultivars were soaked up in refined water for 0, 6, 12, 18, 30 and 48 hrs. After soaking, drying of the seeds was done at 27°C in a constrained air stove for 48 hrs before the L-DOPA test. Use of three replications was done for every action.

Abrasion of seeds

The abrasion of seeds was tested, the treatments were lasted for a time of 0, 5, 10, 20, 30, 40, 50, 100 seconds. In each treatment four seed replications were used and whole abrasion work was done two times (total of eight replications). To know about the quantity of material removed by the abrasion treatment weighing of seeds was done.

When incubation in the L-DOPA solution was made, centrifugation of the samples was made for 1 min in a microcentrifuge for sedimentation of the particulates which caused turbidity of the reaction solution and that is the reason that interference of spectrophotometer absorbance measurement occurred.

Seed size

Seeds were divided into two groups i.e. large sized seeds and small sized. Mature seeds selection was made visually from each of the group. Weighing of seed aliquots was done individually.

It is essential to replace seeds with seeds having large or small size for getting of desired seed weight of

0.23g for large and 0.12 g weight for small seeds. For each treatment three replications were used.

Embryos verses distal seed parts

Cutting of seeds was done to the middle (half of the seed was with embryo portion and distal portion without embryo) and a half seed weight of 0.09 g was used for each of replications for the ten cultivars. Samples were centrifuged for 1 min in a micro centrifuge for sedimentation of the particulates which resulted turbidity of the reaction solution and so it also interfered with the spectrophotometer absorbance.

Light and dark cultured seedlings

Both the light and dark cultured seedlings were placed in positions of equal distances in petri dishes on moderately wet filter paper (3 ml tap water per petri dish of 10 cm diameter).

In each dish 10 seeds each of light and dark culture were placed at 25°C.

Plantlets were raised to measure PPO activity using glass dishes of 15 cm and 3 cm height under day light culture at 25°C.

Results and discussion

Germination conditions

PPO activities correlation to seedling development during germination

PPO activity was observed and studied during whole process of germination (Fig. 1).

Table 1. Analysis of variance for the effect of wheat embryo half vs. distal half seed on polyphenol oxidase (PPO) action determined by L-DOPA assay.

Source	d.f	Mean square	F-value	P-value
Cultivar	10	0.917	133.09	< 0.0001
Seed half	1	0.039	20.3	0.056
Interaction	2	0.008	1.09	0.65
Error	32	0.004		

After the first and the second day of incubation PPO activity was observed in wheat bran. On first day enzymatic activity reached to high level and then started to decrease up to 7th day of germination. In endosperm from 2nd day to 6th day highest activities were recorded while lowering of activity level was noted on 7th day. While PPO activity increase was observed in embryo with the onset of germination up to 7th day. In seedling, enzymatic activity started after 5th day of germination and increased up to 7th day.

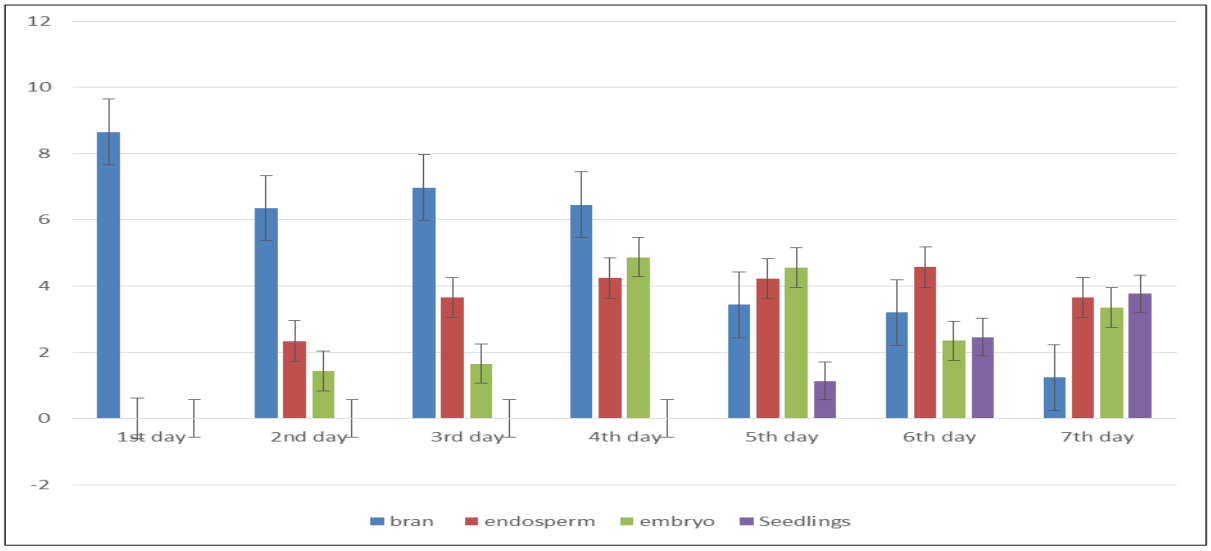

Fig. 1. PPO activities correlation to seedling development during germination.

PPO activities in relation to effect of light and dark on plant development during germination

Noticeable PPO activities were recorded in both germinating seeds plus growing seedlings.

As the process of germination started in the seeds, highest activities of enzyme was noted on the first day of germination and compared both in light and dark (Fig. 2).

The PPO action was monitored both in light presence and dark was also compared. The activities showed a rapid reduction in both light and dark and it was lowest on third day of germination. However PPO action decreased more in the absence of light in the growing seedlings than in the presence of light.

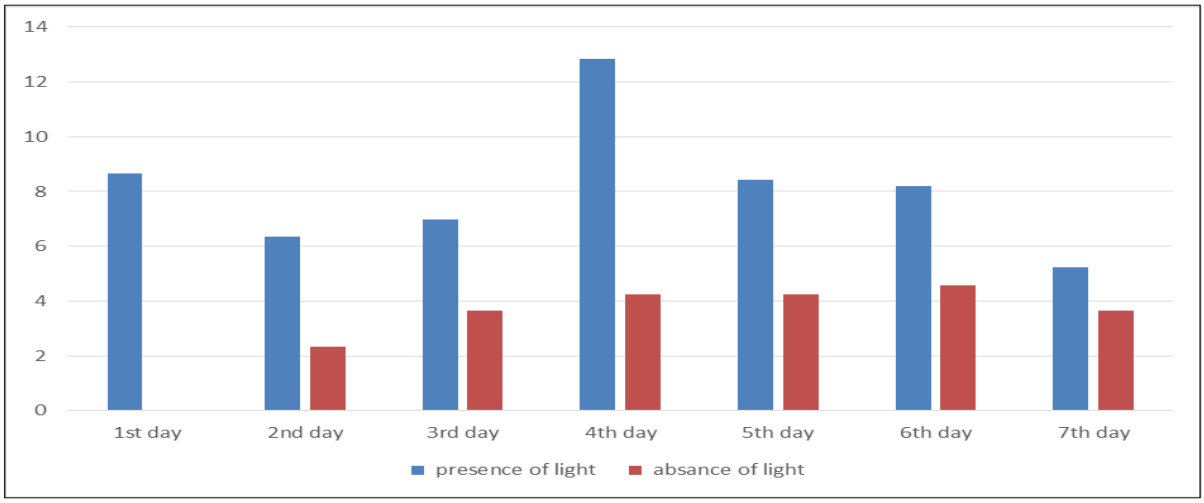

Fig. 2. PPO activities relation to effect of light and dark on seedling development during germination**.**

On day fourth of germination, when new organs were forming in the seedlings, there occurred increased PPO activity in both light plus dark cultures**.**

Incipient germination effect on PPO assay action

For simulation of germination conditions imbibition of seeds was done up to 48 hrs and then were dried. Zero change was observed in enzyme action for seeds in which imbibition was done up to 6 hrs in all the ten cultivars. After that time elevated PPO action was recorded up to 24 hrs. After 24 hrs lowering down in the activity was documented up to 48 hrs of imbibing of the seeds. In all cultivars, shoots and roots were observed to develop after 36 hrs of imbibing, while radical emerged after 24 hrs (Fig. 3).

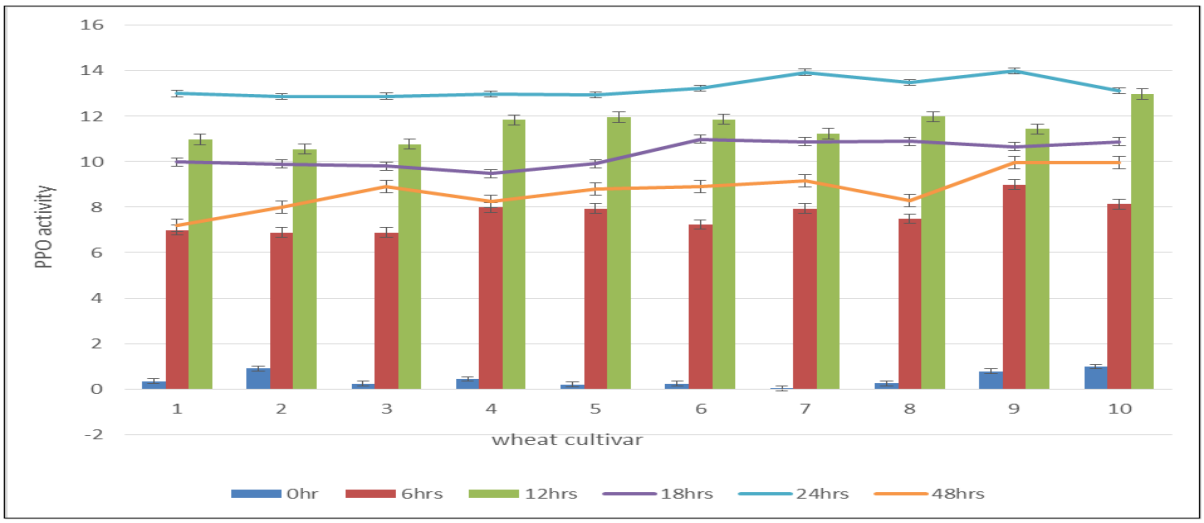

Fig. 3. The effects of incipient germination on PPO activities.

Abrasion of seeds

To find out whether the effect of mechanical damage on PPO activities occurs during storing and handing out, wheat seeds were scraped for time duration of 0 to 100 sec. The scrapping causes removal of parts in grains like pericarp (bran) and then the embryo consistently by the device used in the process. A successive decrease in seed weight and rising in abrasion time was documented (Fig. 4).

Stability of PPO is clear in dry seeds and the minor harm come across during harvest or threshing which does not make significant rise and decline in enzymatic action.

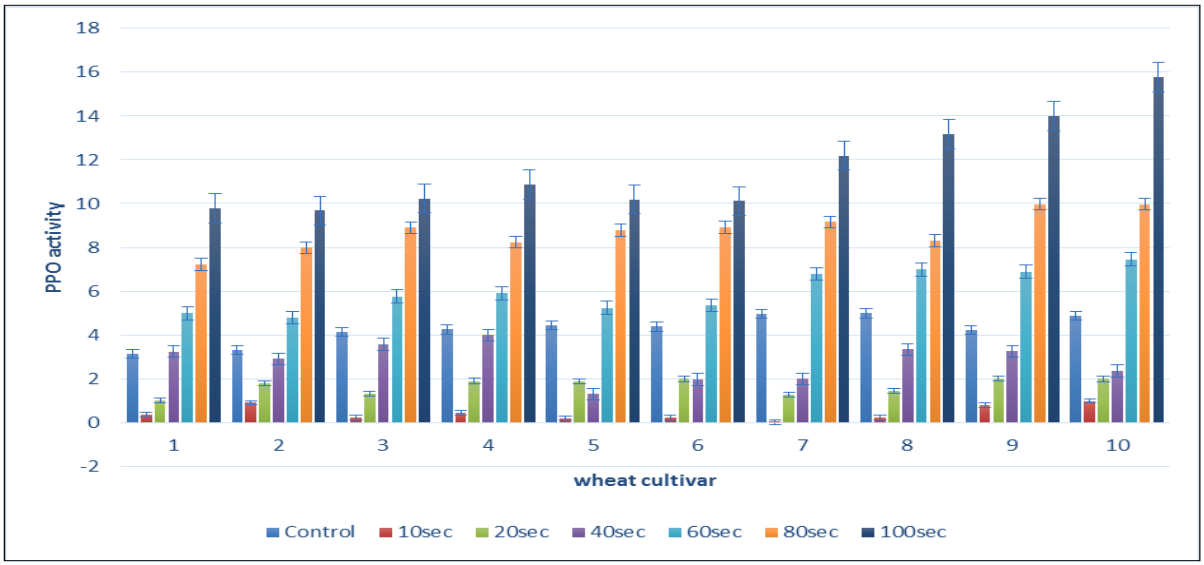

Fig. 4. Effect of abrasion of seeds on PPO activity.

Seed size effect on activity of PPO assay

Measurement of enzyme action was investigated making two groups of seeds i.e groups of small size seeds and large size seeds, all the ten wheat cultivars.

Five seeds of average size from each category were selected (0.12g) for small seeds and 0.23g for large seeds**.** Measurements of enzyme action were investigated using L-DOPA assay. High enzymatic action was noted in large seed than the smaller ones. On the other hand, the extent of the cultivar involvement proved to be greater as compared to seed size (shown in Fig. 5).

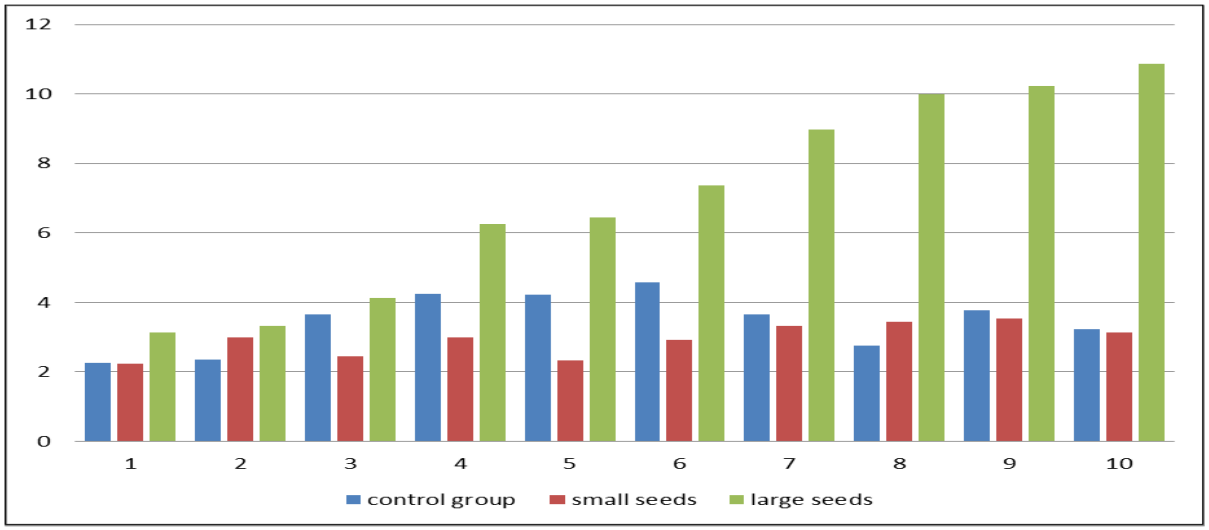

Fig. 5. Effect of seed size on PPO activities.

Comparison of PPO assay activities in embryo verses distal (non-embryo) portions of the seed

The seeds were cut into two halves from corner to corner and comparison was made between their PPO actions. The aim of cutting the seed in two halves is in order to study how mechanical damaged seeds or broken seeds effect PPO activity**.** For this purpose ANOVA was used whether an embryo part is present or not has important insignificant consequences Analysis of variance for the effect of wheat embryo half vs. distal half seed on polyphenol oxidase (PPO) action determined by L-DOPA assay (Table 1).

Discussion

Our study investigated that PPO activity reaches to its highest level during seed germination and same results were given by many other authers likeTaneja and Sachar, 1974; Kocacaliskan *et al*., 1995; Demeke *et al*., 2001; Maki and Moro-hashi, 2006 and Dicko *et al.,* 2006. In first 6 hours there was a slight rise in PPO activity and this was increasing slowly upto 24 hours. However, there occurred a decrease in PPO action upto 48 hours because of imbibing process.

On the other hand imbibing grains of wheat for 36 hrs there was growth of early roots and shoots in all of the cultivars. After 24 hrs of imbibing the grains of wheat, there occurred redical growth. Demeke *et al.* (2001) also gave results similar to these current findings. According to Demeke *et al.* (2001) PPO assay showed no variation for the seeds which absorbed water for 8 hrs.

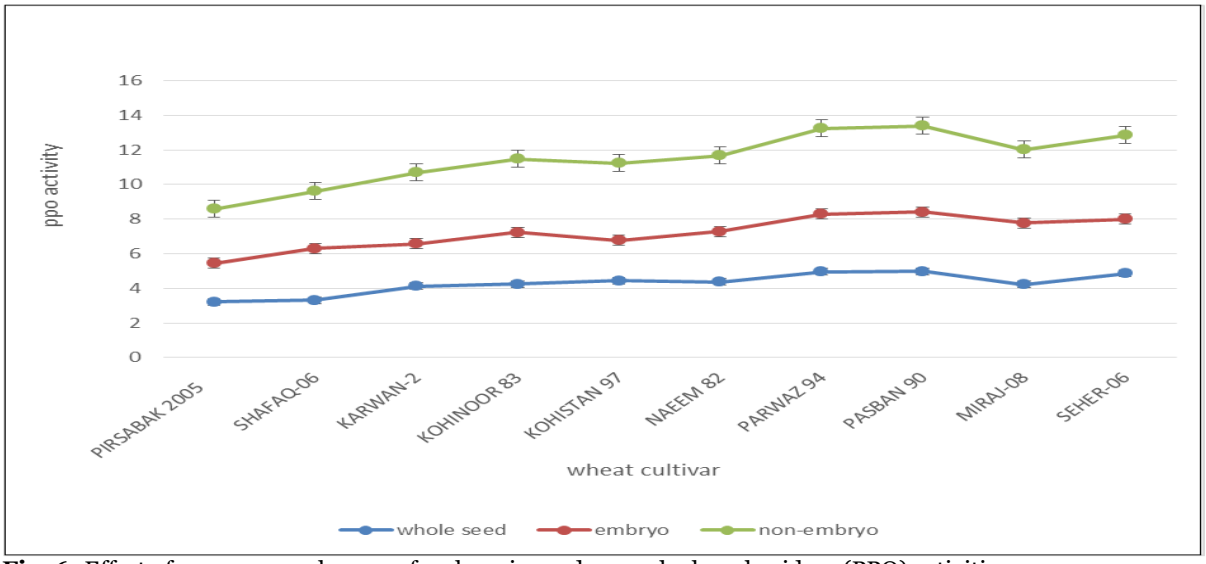

Fig. 6. Effect of presence or absence of embryo in seeds on polyphenol oxidase (PPO) activities.

The current result is different from Dicko *et al.* (2006), whose research was on 50 sorghum varieties gave the result of zero PPO activity in sorghum grains during germination. PPO is found in testa in larger amount, when induced to endosperm and growing seedlings, results showed that this may be the reason of germination and development of new plant and also protecting of embryo. PPO action was greater in embryo than cotyledons and tissues, also in wheat roots plus coleoptiles gave high PPO activity according to Taneja and Sachar, 1974. However, research with 50 sorghum varieties yielded data showing that PPOs were not activated in germinated sorghum grains (Dicko *et al.,* 2006).

For all the ten cultivars there occurred rise in PPO activity while lowering down of time of seed abrasion. Stability of PPO is clear in dry seeds and the minor harm come across during harvest or threshing which does not make significant raise and decline in enzymatic action. Large sized seeds showed greater PPO activity than smaller ones according to the ongoing research study findings of ours, similar result was reported by Demeke *at al.* (2001).PPO high activity for larger sized seeds using substrates as catechol and tyrosine was documented in wheat

grains. Same research results were figured out by Baik *et al.* (1994) about high PPO action in big sized seeds than smaller ones. In their study they used 2.8 mm size seeds having high PPO action than 2.1mm seeds size. Our findings are similar to the studies explained earlier that big size seeds have greater PPO actions as compared with smaller size seeds, but the rise in PPO action in big size seeds is not proportional to the sizes of seeds.

The smaller size seed, which has weight equal to about half than that of the big size seed and was a smaller part of all seeds, has, more than 75% of the PPO assay activities expressed by the bigger seeds.

The effect of light on activity of PPO during seed germination, even though environmental factors such as temperature, length of the day and increase or decrease of moisture showed to have influence on the activity of PPOs .

Acknowledgements

The authors would like to thank to Mr. Syed Zahir Shah for his guidance to carry out the research Work.

References

Baik B, Czuchajowska KZ, Pomeranz Y. 1995. Discoloration of dough for oriental noodles. Cereal Chemistry **72,** 198-205.

Demeke T, Chang HG, Morris CF. 2001. Effect of germination, seed abrasion and seed size on polyphenol oxidase assay activity in wheat. Plant Breeding **120,** 369–373.

Dicko MH, Gruppen H, Zouzouho OC, Traoré AS, Van Berkel WJH, Voragen AGJ. 2006a. Effects of germination on the activities of amylases and phenolic enzymes in sorghum varieties grouped according to food end-use properties. Journal of Science and Food Agriculture **86,** 953-963.

Feillet P, Autran JC, CIcard-Vernière. 2000. Pasta brownness: an assessment. Journal of Cereal Science **32,** 215-233.

Kayani AK, Qureshi S, Kayani WK, Qureshi R, Waheed A, Arshad M, Gulfraz M, Laghari MK. 2010. Assessment of wheat yield potential after cropping mungbean (Vigna radiate (L.) Wilczek). Pakistan Journal of Botany **42(3),** 1535-1541.

Kocacalıs Kan I, Demir Y, Kabar K. 1995. A study on polyphenol oxidase activity during seed germination. Phyton, International Journal of Experimental Botany **35,** 37- 43.

Maki H, Morohashi Y. 2006. Development of polyphenol oxidase activity in the micropylar endosperm of tomato seeds. Journal of Plant Physiology **163,** 1–10.

Mayer AM. 2006. Polyphenol oxidases in plants and fungi: Going places? A review. Phytochemistry **67,** 2318-2331.

Taneja SR, Sacher RC. 1974. Separate monophenolase and o-diphenolase enzymes in *Triticum aestivum*. Phytochemistry **13,** 1367-1376.

Zaini NA, Osman A, Hamid A, Ebrahimpour A, Saari N. 2013. Purification and characterization of membrane-bound polyphenoloxidase (mPPO) from Snake fruit [Salaccazalacca (Gaertn.) Voss]. Food Chemistry **136,** 407-414.