Effect of harvest time and height on seed quality and enzyme activity in onion (*Allium cepa* L.) seeds

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Abstract

Seed development and vigor may be influenced by harvest time and height of harvest in onion seeds. Thus, an experiment was conducted to evaluate the effects of different harvest times and height of harvest in enzymatic activity and biochemical changes of seeds in two growth seasons (2014-2015) at Research Farm of the Seed and Plant Institute, Karaj, Iran. The experimental design was factorial in a randomized complete block with three replications. Four times of harvest namely, T1 (moisture content: 60%), T2 (moisture content: 50%), T3 (moisture content: 39%), and T4 (moisture content: 11%) along with four harvest heights (0, 10, 20, and 30 cm bottom of inflorescence) were randomized to the plots. Seeds were harvested in 10-day intervals at four stages during development and maturity. Results showed in both studied years the maximum catalase (1.847 OD/mg protein min^{-1}) and peroxidase activity (3.852 OD/mg protein min^{-1}) and also the minimum MDA content (0.068 nm g FW) were achieved in the 3rd harvest time and 20 cm height of harvest. In agreement with the achieved result about enzymatic activity, the maximum 1000 seed weigh (4.440 g) was observed in the second year and the third harvest with 20 cm height of harvest. Maximum seed vigor as measured by the electrical conductivity of seed leachate (58.70 µs/cm/g) was obtained at second harvest time with 20 cm height of the harvest. In this study, earlier harvests due to immaturity and later harvests because of aging reduced seed vigor of onion. The best treatment for achieving suitable seeds was the third harvest time and 20 cm height of harvest. Also, biochemical traits were helpful in determining the suitable time of harvesting in onion seeds.

Keywords: catalase, harvest time, onion, peroxidase, vigor

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Introduction

Onion seeds are orthodox seeds, which can be stored for a long time under low-temperature and low-humidity condition (Solberg et al., 2020). In orthodontic seeds, the drying stage plays an important role in completing the seed maturity and preparing them for the germination stage (Angelovici et al., 2010). Physiological maturity is reached when the seed displays maximal dry

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weight accumulation and considerable water loss. Since physiologic maturity is considered as harvesting times to the plants, identification of the physiologic maturity stage is one of the most important times to reduce the loss of products and also to prevent the deterioration of the seed in the field (Patrick and Offler, 2001; Vidigal et al., 2011).

In some studies, seed maturity was determined through physiological indices such as seed weight and moisture (Eskandari, 2012), germination percentage and emergence rate index (Ghassemi-Golezani and Hosseinzadeh-Mahootchy, 2009), the electrical conductivity of seed (Vidigal et al., 2011), and seed and fruit color. Recently, in addition to these indicators, biochemical methods are also used to identify the appropriate harvest time. These methods include cell respiration (Ramya et al., 2012) and reserve mobilization (Oliveira et al., 2013).

Seed deterioration at the end of the growing season are due to a number of factors including lipid peroxidation by free radicals, protein depletion and deactivation of enzymes, cell membrane degradation, and damage to the DNA (Murthy et al., 2003). The degradation and inactivation of enzymes are also a result of alterations in the molecular structure of these enzymes during seed deterioration (Bailly, 2004; Lehner et al., 2008; McDonald, 2004). In most studies, researchers reported reduced activities of superoxide dismutase, catalase, peroxidase, and glutathione reductase enzymes in seeds. Decreased activities of these enzymes reduces respiratory capacity and, as a result, reduces sufficient energy (ATP) and assimilates for germination. Therefore, changes in the molecular structure of the enzymes may reduce the germination traits of the seeds.

Peroxidase and catalase both scavenge oxygen radicals and convert H_2O_2 to oxygen and water. Decreased catalase activity was associated with ageing, accompanied by an increase in lipid peroxidation and loss of vigor and viability in maize (Oliver et al., 1990) and sunflower (Bailly et al., 2002). Peroxidase also plays an important role in maintaining seed quality and inhibiting oxidative damage to seeds (Govindaraj et al., 2017).

Table 1
Meteorological data of Karaj area during growth period of Onion during 2014 and 2015 growing seasons

Months	Means of temperature (°C)		Rainfall (mm)	
	2014	2015	2014	2015
April	15.7	16	10.5	66
May	18.7	21.8	22	14.5
June	24.5	26.3	1.5	5
July	27.1	28.9	0	9
August	25.3	28.7	4	0
September	24.3	24.8	0	0

Today, molecular and biochemical indices are used to identify the best seed harvesting time, reduce harvest losses, increase the storage time of seeds, and ultimately increase seed quality. On the other hand, the use of biochemical markers to select the appropriate harvest time requires more research. In order to use biochemical and molecular indices related to seed quality, the relationship between these indices and seed germination traits should be investigated. In the present study, experiments were conducted to find out possible ways to improve seed biochemical activities with respect to harvest time and height of harvest in onions.

Materials and Methods

A field experiment was conducted at the Research Farm of the Falat Company, Karaj, Iran (latitude 35°48" N, longitude 51°10"E, Altitude 1321 m above mean sea level) in 2014 and 2015. The experimental area is located in the country's semiarid temperate zone (according to the Köppen climate classification), which features cold winters and moderate summers in north-western Iran. The mean annual rainfall is about 124.4 mm, and most rainfall is mainly concentrated between winter and spring (January to June). The meteorological data recorded during the period of onion growing are given in Table 1.

In order to determine the physical and chemical characteristics of experimental field, soil sample was taken two weeks before planting. Table 2 shows the physicochemical characteristics of the farms in the test site.

Table 2
Physical and chemical characteristics of the soil in the experimental location

year	Organic Material (%)	pH	EC $ds.m^{-1}$	Clay (%)	Silt (%)	sand (%	N Total (mg/kg)	P (mg kg)	K (mg/kg)	Fe (mg/kg)	Zn (mg/kg)
2014	0.71	8.3	1.3	33	38	29	0.09	32	161	6.9	2.5
2015	0.69	8.2	1.2	35	37	31	0.10	30	154	6.1	2.4

The experimental design was a randomized complete block arranged as factorial with three replications. Four times of harvest, namely T1 (moisture content: 60%), T2 (moisture content: 50%), T3 (moisture content: 39%), and T4 (moisture content: 11%) along with four harvest heights (0, 10, 20, and 30 cm bottom of inflorescence) were randomized to the plots. Seed moisture was measured by the standard method of high temperature by the oven (High Constant Temperature Oven Method) according to the criteria of the International Seed Testing Association (ISTA, 2012). Times of harvest are presented in Table 3.

Table 3
Times of harvest

Stage	2014	2015
1st harvesting time	9 July	17 July
2nd harvesting time	19 July	27 July
3rd harvesting time	31 July	6 August
4rd harvesting time	10 August	16 August

Onion cultivar Zargan was selected for the study because of its availability in the market. Onion bulbs were purchased from Falat Seed Company, Iran. Recommended dose of fertilizer (150:100:180 kg/ha NPK) in the form of urea, triple super phosphate, and potassium sulfate was applied to grow the crop. Half of nitrogen fertilizer was given simultaneously with phosphorus fertilizer and potash at the time of field preparation and half of the other nitrogen fertilizer at the time of flowering and early seed formation.

After seedbed cultivation with a moldboard plow and two vertical disking in early spring, onion bulbs were sown by hand at the depth of 4 cm on May 5th, 2014 and May 8th. Plant density was 20 plants per m^{-2} in both study years. A drip irrigation system (irrigation water pH of 7.3) was used and all experimental plots were irrigated on weekly basis to replenish the crop evapotranspiration. Irrigation continued according to climatic condition and plant requirements through the growing seasons (2014-2015). Weeds were controlled by hand. The onion seeds were harvested according to mentioned treatments (specific harvest time and height of harvest) in both study years. Umbels were cut by hand with approximately 10, 20, 30 cm of the seed stalk (scape) attached when the seeds reached 60, 50, 39, and 11% moisture content, and moved to the laboratory. The collected samples were kept at the lab for 2-3 weeks. After drying the samples, the seeds were removed from umbels and cleaned. Then, all seed samples were stored in canvas bags at low humidity and low temperature for biochemical and germination studies.

Measurement of Malondialdehyde content

The malondialdehyde (MDA) content of the seeds was measured by the Heath and Parker method (1986). Briefly, 0.5 g seed was homogenized in 5 ml of 0.1% trichloroacetic acid (TCA) and centrifuged at 20,000 × g for 10 min, and MDA content (mM g/FW) was determined by a spectrophotometer at 532 and 600 nm.

Determination of catalase activity

Catalase (CAT) activity was measured 14 days after germination. For this purpose, Karo and Mishra (1976) method was used. The protein extract (60 µL) was added to Tris buffer (50 mM, pH = 7) containing 5 mM H_2O_2 on an ice bath, then the absorbance curve was plotted at a wavelength of 240 nm. Enzyme activity was obtained for OD mg^{-1} protein of the fresh tissue.

Peroxidase assay

Karo and Mishra (1976) was used for assaying of Peroxidase (POD) activity. The protein extract (50

Table 4
Analysis of variance for germination traits of onion affected by harvest time and height of harvest in yeas 2014 and 2015

S.O.V	df	Mean Squares					
		CAT Activity	POD Activity	PPO Activity	MDA Activity	1000 Seed Weight	EC
Year (Y)	1	0.032 **	0.179 **	2.478 **	0.0330 **	8.023 **	1.771
Y (Replication)	4	0.001	0.003	0.0003	0.00016	0.007	1.664
Time (T)	3	0.603 **	29.309 **	0.235 **	0.0139 **	1.007 **	5967.676 **
Y × T	3	0.257 **	0.076 **	0.114 **	0.0013 **	0.479 **	38.213 **
Height (H)	3	0.873 **	1.817 **	0.063 **	0.0044 **	0.291 **	863.386 **
Y×H	3	0.030 **	0.145 **	0.004 **	0.0011 **	0.179 **	5.334
T×H	9	0.037 **	0.394 **	0.039 **	0.0009 **	0.072 **	1173.563 **
Y×T×H	9	0.039 **	0.114 **	0.014 **	0.0005 **	0.088 **	53.545 **
Eb	60	0.0024509	0.003585	0.0000455	0.000156	0.0044 **	2.220
CV (%)	-	4.16	2.46	1.50	9.68	1.65	1.57

* and ** show significant at 5% and 1% probability levels, respectively.

µl) was added to 2.5 ml extraction buffer containing 100 µM Tris buffer 100 mM, hydrogen peroxide 5 mM, and 10 mM pyrogallol in the ice bath, and absorbance changes were read at a wave length of 425 nm. Enzyme activity was obtained for OD µg protein min^{-1} of the fresh tissue.

Polyphenol oxidase assay

Polyphenol oxidase (PPO) activity was measured by Karo and Mishra (1976) method. A protein extract (100 µl) was solved in 1.5 ml Tris 0.2 M and 0.3 ml pyrogallol 0.02 M, and the resulting composition was placed in the Bain-marie bath at 25 °C for five minutes. Then, the absorbance was recorded, and enzyme activity was obtained for OD µg protein min^{-1} of fresh tissue.

Electrical Conductivity

According to ISTA standards, one of the indicators for measuring seed vigor is performing the electrical conductivity (EC) test. The method described by Powel et al. (1984) was used to measure the electrical conductivity of the seeds. For this purpose, two replicates of 100 seeds per each treatment were placed in 250 cc distilled water at 20 °C for 24 hours. Then, the electrical conductivity of each of the replicates was measured with an EC meter and the electric conductivity of each treatment was determined using the following equation:

EC (µs/cm/g) = [(EC1/SW1) + (EC2/SW2)]/2

Statistical Analysis

The data were tested for homogeneity and normality of residuals using the Bartlett and Kolmogorov-Smirnov tests, respectively. A combined analysis of variance (ANOVA) was used to compare interactions for 2 years using PROC GLM, SAS 9.1 software. Mean comparisons were carried out using Duncan test when the F test proved significant at P≤ 0.05.

Results

All studied traits were affected by interaction of year × harvest time × height of harvest (P≤0.01) (Table 4).

In both years of the study, maximum catalase activities (1.847 and 1.773 OD/mg protein min^{-1}, respectively) were found in the 3rd harvest time and 20 cm height of harvest (Table 5). Also, in all harvest times maximum catalase activities were achieved in 20 cm height of harvest. It seems that translocation of substances from green parts of fruit increased the biochemical activities of seeds in the end of seed filling period (Table 5). Similarly, peroxidase activity increased dramatically in both years in the 3rd harvest time and 20 cm height of harvest. The differences between minimum and maximum activities of catalase and peroxidase were 144.31% and 198.37%, respectively (Table 5). The maximum peroxidase activity (3.852 OD/mg protein min^{-1}) was achieved in second year with the 3rd harvest time and 20 cm height of harvest. In contrast, the minimum peroxidase

Table 5. Comparison of means for germination traits of onion affected by harvest time and height of harvest in 2014 and 2015 years.

	Treatments		CAT (OD/mg protein min^{-1})	POD (OD/mg protein min^{-1})	PPO (OD/mg protein min^{-1})	MDA (nm g FW)	1000 seed weight (g)	EC (µs/cm/g)
2014	Harvest Time 1	H0	0.756 ^{q}	1.371 ^{nop}	1.107 ^{x}	0.149 ^{bc}	2.843 ^{n}	127. 7 ^{b}
		H10	0.938 ^{no}	1.627 ^{ij}	1.142 ^{w}	0.137 ^{cd}	3.203 ^{l}	126.1 ^{b}
		H20	1.008 ^{mn}	1.631 ^{ij}	1.228 ^{t}	0.099 ^{hij}	3.464 ^{k}	108.1 ^{d}
		H30	0.873 ^{op}	1.506 ^{klm}	1.325 ^{q}	0.135 ^{cdef}	2.477 ^{k}	105.9 ^{d}
	Harvest Time 2	H0	1.179 ^{jk}	1.757 ^{h}	1.205 ^{u}	0.134 ^{cdef}	3.062 ^{k}	103.3 ^{e}
		H10	1.274 ^{efghi}	3.524 ^{cd}	1.319 ^{q}	0.112 ^{fghi}	3.766 ^{j}	95.73 ^{ij}
		H20	1.336 ^{de}	3.573 ^{c}	1.261 ^{s}	0.0986 ^{hij}	3.927 ^{hi}	59.73 ^{ij}
		H30	1.223 ^{ghij}	3.505 ^{cd}	1.301 ^{r}	0.121 ^{defgh}	4.038 ^{fgh}	69.70 ^{r}
	Harvest Time 3	H0	1.199 ^{hijk}	3.013 ^{f}	1.177 ^{v}	0.112 ^{efghi}	3.968 ^{fghi}	84.73 ^{n}
		H10	1.319 ^{def}	3.738 ^{b}	1.166 ^{v}	0.080 ^{jk}	4.066 ^{efgh}	60.83 ^{r}
		H20	1.847 ^{a}	3.800 ^{ab}	1.424 ^{l}	0.068 ^{k}	4.080 ^{def}	91.10 ^{lm}
		H30	1.219 ^{hij}	3.430 ^{d}	1.110 ^{x}	0.105 ^{ghi}	3.992 ^{fghi}	96.57 ^{ghij}
	Harvest Time 4	H0	1.011 ^{mn}	1.384 ^{nop}	1.218 ^{t}	0.118 ^{defgh}	3.878 ^{ij}	98.57 ^{fgh}
		H10	1.446 ^{bc}	1.456 ^{lmn}	1.353 ^{p}	0.098 ^{hij}	3.991 ^{fghi}	90.13 ^{lm}
		H20	1.458 ^{bc}	1.528 ^{jkl}	1.391 ^{n}	0.089 ^{ijk}	3.946 ^{ghi}	97.47 ^{fghji}
		H30	1.242 ^{fghij}	1.386 ^{nop}	1.381 ^{no}	0.107 ^{ghi}	3.878 ^{ij}	99.23 ^{fg}
2015	Harvest Time 1	H0	0.990 ^{mn}	1.406 ^{mno}	1.406 ^{m}	0.210 ^{a}	4.081 ^{def}	131.4 ^{a}
		H10	1.186 ^{ijk}	1.623 ^{ij}	1.463 ^{j}	0.208 ^{a}	4.170 ^{cde}	122.1 ^{c}
		H20	1.312 ^{defg}	1.688 ^{hi}	1.443 ^{k}	0.138 ^{cd}	4.247 ^{bc}	108.5 ^{d}
		H30	1.005 ^{mn}	1.591 ^{ijk}	1.563 ^{g}	0.190 ^{a}	4.357 ^{ab}	106.1 ^{d}
	Harvest Time 2	H0	0.827 ^{pq}	2.792 ^{g}	1.377 ^{o}	0.168 ^{b}	4.360 ^{ab}	92.67 ^{kl}
		H10	1.192 ^{ijk}	3.526 ^{cd}	1.404 ^{m}	0.192 ^{a}	4.403 ^{a}	95.77 ^{ij}
		H20	1.510 ^{b}	3.600 ^{c}	1.410 ^{m}	0.131 ^{cdef}	4.263 ^{bc}	58.70 ^{r}
		H30	1.061 ^{lm}	3.304 ^{e}	1.494 ^{i}	0.132 ^{cdef}	4.193 ^{cd}	81.63 ^{o}
	Harvest Time 3	H0	1.119 ^{kl}	3.306 ^{e}	1.727 ^{c}	0.104 ^{hi}	4.367 ^{ab}	89.30 ^{m}
		H10	1.382 ^{cd}	3.787 ^{ab}	1.695 ^{e}	0.116 ^{defgh}	4.407 ^{a}	63.93 ^{q}
		H20	1.773 ^{a}	3.852 ^{a}	1.707 ^{d}	0.120 ^{defgh}	4.440 ^{a}	96.47 ^{hij}
		H30	1.289 ^{efgh}	3.566 ^{c}	1.517 ^{h}	0.114 ^{defg}	4.260 ^{bc}	97.80 ^{fghi}
	Harvest Time 4	H0	0.866 ^{op}	1.291 ^{p}	1.648 ^{f}	0.136 ^{cde}	4.240 ^{bc}	99.43 ^{f}
		H10	0.889 ^{op}	1.406 ^{mno}	1.653 ^{f}	0.129 ^{cdefg}	4.340 ^{ab}	88.87 ^{m}
		H20	1.317 ^{def}	1.564 ^{jk}	1.967 ^{a}	0.133 ^{cdef}	4.343 ^{ab}	94.80 ^{jk}
		H30	1.024 ^{mn}	1.308 ^{op}	1.774 ^{b}	0.136 ^{cdef}	4.360 ^{ab}	91.97 ^{l}

* and ** show significant at 5% and 1% probability levels, respectively. H0, H10, H20, and H30 denote height of harvest at 0, 10, 20, and 30 cm, respectively.

activities were observed in the first and last harvest times (Table 5).

The maximum polyphenol oxidase activity (1.967 OD/mg protein min^{-1}) was achieved in the second year and last harvest with 20 cm height of harvest. Overall, polyphenol oxidase activities in the second year and especially in the third and fourth harvests were higher than in other harvests (Table 5).

MDA content decreased with increasing the antioxidant enzymes activity. The minimum MDA content was observed in the first year with the third harvest and 20 cm height of harvest (Table 5).

In agreement with the findings of the study on enzymatic activities, maximum 1000 seed weight was observed in the second year and the third harvest with 20 cm height of harvest (Table 5). In addition, seed weight in the second year was higher than that in the first year.

Investigation of electrolyte leakage is a very important parameter, which is investigated for determining the health of membrane in the plant cells. In both studied years at the first harvest time, electrical conductivity was higher than the other harvest times. Maximum electrical conductivity was observed in the second year with the first harvest time and zero height of harvest. However, the minimum electrical conductivity was achieved in the second year, the second harvest

Table 6. Correlation matrix between different parameters of onion seeds

	CAT Activity	POD Activity	PPO Activity	MDA	1000 seed weight	EC
Catalase activity	1					
Peroxidase activity	0.53824 **	1				
Polyphenol oxidase	0.15427 ns	-0.03838 ns	1			
MDA	-0.46611 **	-0.31709 **	0.13689 ns	1		
1000 seed weight	0.31354 **	0.33444 **	0.68873 **	0.11744 ns	1	
EC	-0.42195 **	-0.64479 **	-0.12229 ns	0.49370 **	-0.41728 **	1

* and ** represent significant at 5% and 1% probability levels, respectively.

time, and 20 cm height of harvest. In most cases, the maximum electrical conductivity of seeds were recorded with zero height of harvest (Table 5).

Correlation matrix among the traits under study is presented in Table 6. A positive and significant correlation was observed between enzymes activity and 1000 seed weight as an indicator of seed quality. Also, catalase and peroxidase activity had negative correlation with EC which was another character of seed quality.

Discussions

DemirKaya et al. (2010) reported that with a decrease in germination percentage, catalase activity also decreased in seeds of onion cultivars. This is in agreement with a number of studies, in the literature, e.g. Basra and Malik (1994) on onion seeds, Bailly and coworkers (Bailly et al., 2002)on sunflower seeds, Goel et al. (Goel et al., 2003) on cotton seeds, and some researchers (XiangYue et al., 1998) on cucumber seeds. Catalase activity decreased with decreasing seed germination in pepper seeds (Demirkaya, 2013). Seed deterioration leads to biochemical and enzymatic changes and seedling growth in seed (Kapilan, 2015). In line with these reports, previous studies on beech (Pukacka and Ratajczak, 2005), onion (Rao et al., 2006), safflower (Zamani et al., 2010), and maize [(Kapilan, 2015) and (Mansouri-Far et al., 2015)] seeds showed that ageing is associated with a reduction in antioxidant enzyme activity.

In our study, the difference between minimum and maximum activities of catalase was 144.31% while for peroxidase the difference was 198.37%. This suggests susceptibility of peroxidase to different harvest time and height of harvest in comparison with catalase activity.

Rao et al., (2006) reported that by reducing the activity of antioxidant enzymes, the amount of peroxidation of unsaturated fatty acids increases, which ultimately leads to an increase in MDA content. Negative correlation between enzymatic activity and MDA was reported by Zamani et al., (2010). DemirKaya (2013) reported when the viability level of seeds dropped, MDA content increased. The results obtained from this study are parallel to the studies of Goel et al. (2003) on cotton seeds, DemirKaya and Sivritepe (Demirkaya and Sivritepe, 2011) on onion seeds, DemirKaya (2013) on pepper and Kaewnaree et al. (Kaewnaree et al., 2011)on sweet pepper seeds.

Seeds reach the physiological maturity when the final dry weight is maximum and the moisture content in the seed is at its lowest level (Bewley et al., 2012). Ghassemi-Golezani et al. (GHASSEMI-GOLEZANI et al., 2016)suggested that mass maturity in safflower cultivars was achieved at about 40 days after flowering with 15-20% seed moisture content.

Patrick and Offler (2001) stated the reason for an increase in leakage in the early stage of seed development is the increased ions and amino acids in seed apoplast, which usually enter the phloem of the mother plant to apoplast seed. In this manner, membrane integrity is mainly verified in onion seeds until the first to fourth harvest time. Earlier harvests due to immaturity and later harvests because of aging reduced seed vigor of onions under all heights of harvest (Table 5). Reduced electrical conductivity of the seeds has been reported around the final seed development stages in fava bean (Ghassemi-Golezani and Hosseinzadeh-Mahootchy, 2009), pepper (Silva et

al., 2015; Vidigal et al., 2011)), and onion (Ramya et al., 2012).

Kavak et al. (Kavak et al., 2012)reported that early and late harvests decrease physical quality and physiological quality of seeds. Mehta et al. (1993) asserted that cultivars of chickpea attained maximum dry matter accumulation (physiological maturity) at second harvest time.

Conclusion

The results obtained from our study suggest that antioxidant capacity is important in onion seeds, and that it is also important to keep this capacity intact. However, determination of harvest time and suitable height of harvest can be an important factor for reducing MDA content and EC levels and increasing enzyme activities to producing proper seeds in onion. The best treatment for achieving suitable seeds was third harvest time with 20 cm height of harvest. Also, the result showed biochemical traits, including enzymatic activity and physiologic traits, were helpful in determining the suitable time of harvest in onion seeds.

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