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ACQUISITION OF DESICCATION TOLERANCE IN FABA BEAN (*VICIA FABA*) AS AFFECTED BY MATURITY STAGES, HARVESTED ORGAN TYPE, AND DRYING METHOD

ALI MORADI[1*] AND EZATOLLAH KARIMI ARPANAHI[1]

[1]*Department of Agronomy and Plant Breeding, Yasouj University, Yasouj, Iran*
[*]*Corresponding author's email: amoradi@yu.ac.ir*

Abstract

A field experiment was carried out to investigate the effects of harvesting organ type and drying method on the acquisition of desiccation tolerance during different development stages of faba bean (cv. Shami) seed. The plants were harvested in three pod developmental stages including Mid-Full Sized Seeds (MS), Full-Sized Seed (FS), and Greenish-Yellow pod (GY) were divided into three organ forms (depodded, podded detached, and podded attached to the plants). Finally, they were dried using two methods including shade-drying and sun-drying. An extra harvesting was also done at yellowish-brown (YB) stage according to the conventional harvest of local farmers as a control. The results showed that as the seeds become more mature, from MS stage to GY stage, germination percentage (GP), natural seedling percentage (NS), content of soluble sugars (SS), and activity of catalase (CAT) and glutathione (GR) enzymes was increased while the mean germination time (MGT) and ascorbate peroxidase (APX) was decreased. From GY stage to YB stage, MGT and soluble protein (SP) were increased while other were traits decreased. In MS, FS, and GY stages, the highest and lowest GP, NS, SS, CAT, and GR enzymes activity were observed in the podded attached shade-dried seed and depodded sun-dried seed treatments, respectively. We found that among all treatments, MGT had an opposite behavior. Overall, the seeds showed desiccation tolerant behavior, a higher amount of SS, CAT, and GR, and lower APX activities compared to those of sensitive seeds. Therefore, it was concluded that harvesting, organ type, and drying method play important roles in the acquisition of desiccation tolerance and promoting seed quality, especially in the early stages of maturity of faba bean seeds.

Key words: Antioxidant enzyme, Drying method, Faba bean, Seed germination, Seed maturity.

Introduction

Faba bean (*Vicia faba*) is the fourth most important legume in the world and is mainly cultivated as a source of protein for human and domestic animal and birds in Europe, North Africa, and East and West Asia (Rowland & Fowler, 1977). This plant is an indeterminate plant that its seeds grow like other orthodox seeds such as soybeans and beans. At the end of the reserves accumulation phase, the orthodox seeds enter the final phase of seed development, called as desiccation phase, which is accompanied by the loss of a considerable amount of water (Kermode, 1995). This stage is a planned pattern for ending the seed development, which acts as a bridge between maturation and germination (Angelovici *et al.*, 2010). As one of their most important characteristics, desiccation tolerance guarantees the survival of orthodox seeds during desiccation, drying, and storage. Desiccation tolerance is a prerequisite for post-harvest germination or artificial drying and is necessary for the switch from development mode to germination (Leopold, 1990; Nedeva & Nicolova, 1997).

Initiation of desiccation tolerance depends on the species, the development stage at which the seed is harvested (harvesting time), and the drying rate of the seed (Black *et al.*, 1999). According to Harington's (1972) hypothesis, the maximum seed quality is obtained at the end of the seed filling stage and then decreases due to delayed harvesting and the onset of seed aging processes on the mother plant. This hypothesis has been confirmed in various plants such as soybeans (Tekrony *et al.*, 1984), beans (Bailly *et al.*, 2001) and maize (Ghasemi-Golezani *et al.*, 2011a). However, some researchers stated that the maximum seed quality was obtained later (Sanhewe & Ellis, 1996; Lehner *et al.*, 2006; Ghasemi-Golezani & Mazloomi-Oskooyi, 2008)). It has been reported that desiccation tolerance in cereals such as wheat, barley, and corn embryos is reached in the early stages of accumulation of reserves. During these stages, seeds are completely tolerant to fast drying (Black *et al.*, 1996; Brenac *et al.*, 1997; Lahner *et al.*, 2006) while the tolerance to fast drying for the seeds of some legumes occurs at the desiccation phase (Sanhewe & Ellis, 1996). In contrary, Huang & Sang (2013) stated that the onset of germination capability in dry corn seeds is related to the time of acquisition of desiccation tolerance (32-36 days after planting; DAP) and the completion of this process affects the maximum germination of dry seeds (52 DAP). Eskandari (2012) reported that the maximum seed quality of cowpea was obtained in the physiological maturity stage (56-52 DAP); i.e., 8-16 days after mass maturity.

Seed drying rate, as an important factor for desiccation tolerance initiation (Nedeva & Nikolava, 1997), is controlled by different drying methods and may lead to changes in the acquisition of desiccation tolerance. It has been reported that slow drying helps start the desiccation tolerance and enhances germination capacity in immature seeds of wheat (Samarh 2010), soybean (Black *et al.*, 1999; Ennen, 2011), and peas (Corbineau *et al.*, 2000). However, these immature seeds could not tolerate fast drying and may tolerate extreme desiccation only at the desiccation phase (Hay & Probert, 1995).

Carbohydrate metabolism and seed tissue capacity of scavenging active oxygen species (AOS) are effective processes in seed germinability. It has been shown that the accumulation of non-reducing sugars,

including raffinose family oligosaccharides (RFO) and sucrose, are associated with acquisition of desiccation tolerance (Lehner *et al.,* 2006; Black *et al.,* 1996; Obendorff., 1997; Corbineau *et al.,* 2000; Bailly *et al.,* 2001). Verdier *et al*., (2013) reported that desiccation tolerance of alfalfa was associated with genes related to LEA proteins, heat shock proteins, and increasing the content of soluble sugars, including, raffinose, verbascose, and stachyose. The accumulation of these sugars depends on the drying rate so that the slow drying of the seeds results in more accumulation of these sugars and, consequently, increased desiccation tolerance and thus germinability (Corbineau *et al.,* 2000). It has been reported that oligosaccharides play a role in the membrane stability and the preservation of cellular integrity during desiccation through the formation of a glass form (Koster & Leopold, 1988), as an index of seed vigor and storage capability (Obendorf, 1997) and a source of energy production during germination and inhibition of some of controllers of hydroxyl radicals (Hicha *et al.,* 2003).

Oxidative processes are produced in a wide range of stresses including desiccation (Moller, 2001) and cause lipid peroxidation, protein oxidation, and DNA damage, which eventually result in cell death (Smith & Berjak, 1995; Leprince *et al.,* 1994). The ability to resist the seeds against desiccation can be related to their ability to scavenge AOS to prevent destructive events caused by these compounds (Vertucci & Farrant, 1995). Such an ability is associated with the cooperation of enzymes such as superoxide dismutase (SOD), catalase (CAT), the glutathione-ascorbate cycle enzymes as well as antioxidant compounds such as glutathione and ascorbate (Scandalios, 1997). It has been reported in sunflower that CAT enzyme activity is increased during seed development and has a close relationship with seed moisture content (Bailey *et al.,* 2004). Moreover, in wheat (Gara *et al.,* 2003), corn (Huang & Sung, 2013), and beans (Bailly *et al*., 2001), ascorbate peroxidase (APX) activity is decreased and catalase and glutathione reductase (GR) are increased during desiccation.

Due to the indeterminate growth in faba bean, seed quality is affected by harvesting the seeds with the different qualities as the result of different physiological statuses, harvesting stage, harvesting type, and drying method. Therefore, the aims of the present study were (1) to investigate the changes in germinability and desiccation tolerance of faba bean seeds harvested at different maturity stages, (2) to find out whether the metabolism of sugars, proteins, and antioxidant defense system play a role in this phenomenon, and 3) assess the effect of the type of harvested organ and drying methods on these mechanisms and some related processes.

Materials and methods

This research was carried out in the laboratory of Seed Sciences and Technology, Faculty of Agriculture, Yasouj University, Iran in 2014. Seeds used in this study were manually harvested in a pod development stage from plants growing from an agricultural farm located in Shushtar (longitude of 48^{o} 20' E, the latitude of 32^{o} 26' N and altitude above 150 m above sea level), Iran. The average annual rainfall in this region is 322 mm that makes it as a semi-arid area. Irrigation, fertilization, weeds control, and plant protection were performed to ensure optimum plant growth. As shown in Table 1, the seeds were harvested in three pod development stages, including 'Mid-Full Sized Seeds (MS)', 'Full-Sized Seed (FS)', and 'Greenish-Yellow pod (GY)' and separated to three organ forms (Depodded, Podded detached, and Podded attached to the plants), and were dried using two methods (shade-drying and sun-drying). An extra harvesting also was done at Yellowish-Brown pod stage (YB) according to the conventional harvest of local farmers (podded attached and sun-dried) and was considered as a control.

In order to perform the treatments, the harvesting and drying were done as follows: For the 'depodded', harvesting organ type a 100- m^2 plot (approximately 2000 seeds) was harvested, all pods containing seed were isolated, and the seeds were removed manually. The seeds were randomly divided into two parts: 1000 seeds for shade-drying (room condition) and 1000 seeds for the sun-drying condition. Also, for the 'podded detached', the same number of seeds were harvested form the pods containing seeds and then were dried through shade-drying and sun-drying. Moreover, in the harvesting organ type of 'podded attached to the plants', plants containing pods were harvested and dried. After reducing moisture content below 10%, the seeds were removed from the drying bed (and removed manually from the pod if necessary), sealed in moisture-proof plastic bags, and were kept at 5^{o}C until the time of standard germination test.

Table 1. Specification of faba bean pods at different harvesting stages.

Harvesting time	Developmental stages	Pod description
First harvesting time (84 DAF)	Mid-Full Seeds (MS)	Pods were green. Seeds filled half of the pod cavity.
Second harvesting time (96 DAS)	Full-sized seed (FS)	Pods were green. Seeds filled the whole pod cavity.
Third harvesting time (108 DAF)	Greenish-yellow pod (GY)	Pods were greenish yellow. Seeds filled the whole pod cavity.
Fourth (125 DAF)	Yellow pod (Y)	Pods were yellow. Seeds filled the whole pod cavity.

Determination of seed moisture content, seed dry weight, and standard germination test: In each developing stages, four replicates of 5 seeds (fresh seed) were randomly selected and seed moisture content and seed dry weight was determined (Anon., 2010).

Standard germination test was performed for each treatment with 5 replicates of 20 seeds (Anon., 2010) using pleated paper (PP) method at 25ºC for 14 days. At the end of the experiment, the germination percentage, mean germination time (equation 1), and the natural seedling percentage were measured.

Equation 1 (Ellis & Roberts, 1981) $MGT = \frac{\sum NiDi}{N}$

Ni = germinated seeds in the i^{th} day, Di = number of days after germination, and N = total number of germinated seeds.

Measurement of biochemical indices: To measure the biochemical indices at each stage, freshly harvested and dried seeds were used. After harvesting, the freshly harvested seeds were immediately packed in an anti-moisture bag and kept at -40ºC until the measurements. The measurements were performed with the embryos isolated from these seeds. In dry seeds, the embryos were separated from imbibed seeds.

Determination of the content of soluble sugars: Soluble sugars were extracted and measured according to the procedure described by Irigoyen *et al.,* (1992). For this purpose, 5 to 10 embryonic axes were isolated from the imbibed seeds (about 0.25 g fresh weight) and were ground using 2.5 ml of 95% ethanol and then transferred to the test tubes and vortexed for 30 seconds. The remaining solid was re-ground by adding 5 ml of 75% ethanol and vortexed. The extract was centrifuged for 30 minutes at 3500 rpm at 25ºC (Sigma 2-16KC, Germany) and the supernatant was used to measure soluble sugars. Finally, using anthrone and sulfuric acid 72%, the absorbance was recorded at 625 nm and soluble sugars content was calculated using the standard curve.

Determination of the content of soluble proteins: Soluble protein was first extracted from the isolated embryonic axis of imbibed seeds according to the method proposed by Dean (1985). Isolated embryos (0.066 g fresh weight) were ground using 2 ml of 50 mM potassium phosphate buffer (pH=6.8) containing 0.1 M KH_2PO_4 and 0.1 M NaOH in the ice bath and then centrifuged for 30 minutes at 12000 rpm at 4ºC. After centrifugation, the upper phase of the extract was transferred to another microtube and used to determine the content of soluble proteins. The concentration of extracted proteins was determined using the Bradford (1976) method at of 595 nm and calculated using the standard curve.

Determining the activity of antioxidant enzymes: Soluble protein extract was used to measure the activity of CAT and APX enzymes.

CAT: The CAT enzyme activity was measured by direct measurement of H_2O_2 at 240 nm, according to Cacmak & Horst method (1991). The reaction mixture contained 2.8 ml of 25 mM sodium phosphate buffer (pH = 6.8), 100 µl of 30 mM H_2O_2, and 100 µl of enzyme extract in a final volume of 3 ml. The activity of the enzyme was expressed as mmol per gram of fresh weight of the embryo axis per minute.

APX: APX enzyme activity was measured according to Nakano & Asada's (1981) method. The reaction mixture with a total volume of 3 ml contained 2.490 ml of 50 mM potassium phosphate buffer with pH 7, 300 µl of 0.5 mM ascorbate, 30 µl of 0.1 µm EDTA, 150 µl of enzyme extract, and 30 µl of 30 mM H_2O_2. The activity of the APX was determined by the decrease in ascorbate absorbance at 290 nm and expressed as absorbance per minute for mmol per gram fresh weight of the embryonic axis.

Glutathione Reductase: GR activity was measured according to the method proposed by Kingston-Smith *et al.,* (1999). Protein extraction from isolated embryo axes was done using 100 mM of potassium phosphate buffer (pH=7.5) containing 0.5 mg EDTA, as performed previously. GR enzyme activity was determined by tracking the NADPH oxidation rate at 340 nm. The final reaction mixture was a 2 ml solution containing 100 mM potassium phosphate buffer containing 1 mM EDTA (pH = 7.5), 3 mM oxidized glutathione (GSS), 0.05 mM NADPH, and 100 µl enzyme extract. The activity of the enzyme was expressed as mmol per gram fresh weight of the embryonic axis per minute.

Statistical analysis

Statistical analysis was carried out using completely randomized design by SAS statistical software. Comparison of the mean was performed using LSD test at 5% statistical level.

Results

The results of analysis of variance (ANOVA) of different treatments were statistically significant for all germination and biochemical traits at 1% probability level (results are not shown).

Dry weight and seed moisture content: Changes in seed moisture content had a negative relation with dry weight during seed growth (Fig. 1). The moisture content (based on fresh weight) of seed in MS, FS, GY, and YB stages was 77.56, 62.24, 47.62, and 21.13 %, respectively. The maximum dry weight of the seeds occurred at the GY stage (96 DAP), so the end of accumulation of food storage or weight maturity occurred at this stage, and then the seed moisture content reached the lowest level in the YB stage (Fig. 1).

Germination percentage (GP): In the early stages of seed maturity (stages of MS and FS), drying method and harvested organ type significantly affected the GP, so that the GP of podded attached shade-dried seeds (89% in the MS stage and 96% in the FS stage) or the podded detached shade-dried seeds (60% in the MS stage and 95% in the FS stage) were higher than the depodded shade-dried seeds (19% in the MS stage and 84% in the FS stage). In

comparison, the lowest amount was observed in sun-dried seeds, especially depodded dried seeds (1% in MS stage and 22% in FS stage). At GY stage, the highest GP was observed in podded attached shade-dried treatment (99%) and it was significantly improved in sun-dried treatments so that there was no significant difference between drying treatments. GP was also decreased to a certain degree (89%) in YB stage (conventional harvesting) compared to GY stage (Table 2).

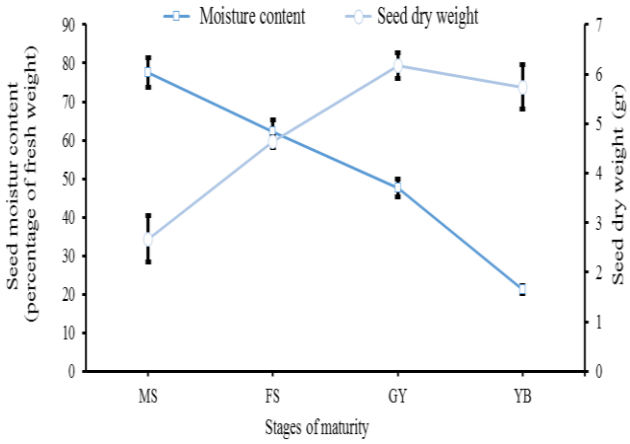

Fig. 1. Changes in the seed moisture content and dry weight of faba bean seeds harvested at different developmental stages. Vertical lines on the points indicate the standard error.

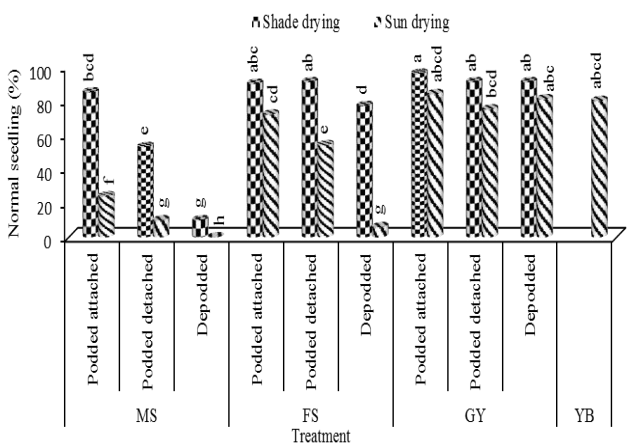

Fig. 2. Mean comparison of organ harvesting type and drying methods at different maturity stages of faba bean for NS (%). Each data is average of five replications. Mean comparison was conducted by LSD test at and bars with common letters are not significantly different 5% statistical level.

Normal Seedlings (NS): In the MS stage, the highest and lowest NS were achieved in the podded attached shade-dried seeds (85%) and depodded sun-dried seeds (0%), respectively; the value for these treatments were 96% and 22% in the FS stage, and 99% and 95% in the GY stage, respectively. However, in the FS and GY stages, there was no significant difference between treatments of podded attached shade-dried seeds and podded detached shade-dried seeds. In the YB stage, the NS was decreased slightly compared to the GY stage, and there was no significant difference among the treatments of this stage (Fig. 2).

Mean germination time (MGT): In the case of MGT, the highest MGT in MS stage was observed in the podded detached sun-dried seeds (5.29 day) while the lowest one was observed in the podded attached shade-dried seeds (3.90 days). However, this index was insignificant (0 days) in the depodded sun-dried seeds because germination did not occur in this treatment. In the FS stage, the value of this index was 3.87 for podded attached shade-dried seeds and 5.50 for sun-dried depodded seeds, respectively. MGT was decreased during development stages of seed and reached its lowest level in GY stage (3.53 days for podded attached shade-dried seeds), so there was no significant difference between these treatments. Afterward, it was slightly increased in the YB stage (4.06 day), but there was no significant difference between GY stage treatments and most of the FS stage treatments (Fig. 3).

The content of soluble sugars (SS): In MS, FS, and GY stages, SS content was significantly higher in the podded attached shade-dried seeds (45.88, 65.5, and 70.19 mg.gr^{-1} fresh weight of the embryonic axis, respectively) compared to depodded shade-dried seeds (31.5, 40.60, and 63.08 mg.gr^{-1} fresh weight of the embryonic axis, respectively) and depodded sun-dried seeds (16.16, 26.38, and 53.65 mg.gr^{-1} the fresh weight of the embryonic axis, respectively). On the other hand, the accumulation of these sugars was not significant in the podded detached shade-dried seeds compared to that of the depodded shade-dried seeds. The highest amount of SS was observed in the podded attached shade-dried seeds at GY stage (19.19 mg.gr^{-1} fresh weight of the embryonic axis), which had a significant difference with other treatments. As shown in Fig. 4, SS was significantly decreased in the YB stage compared to the GY stage treatments (49.05 mg.gr^{-1} fresh weight of the embryonic axis). Both in the dried (shade and sun-dried) and fresh seeds, SS content reached the highest level at GY stage and then decreased at YB stage. However, its value was higher in fresh seeds (Fig. 5).

Catalase (CAT): In MS stage, the highest activity of CAT enzyme was observed in podded attached shade-dried seeds (42.42 mmol.gr^{-1} fresh weight of embryonic axis min^{-1}), which was significantly increased in FS stage (58.07 mmol.gr^{-1} fresh weight of embryonic axis min^{-1}), but there was no significant difference with podded detached shade-dried seeds (52.81 mmol.gr^{-1} fresh weight of embryonic axis min^{-1}) (Fig. 6).

In the early stages of seed maturity (MS and FS stages), the lowest activity of CAT was related to depodded sun-dried seeds (14.35 and 21.53 mmol.gr^{-1} fresh weight of embryonic axis min^{-1}, respectively). However, the activity of this enzyme was higher at the GY stage and the highest amount was seen in depodded shade-dried seeds (69.99 mmol.gr^{-1} fresh weight of embryonic axis min^{-1}). The activity of CAT in the YB stage was also significantly decreased (Fig. 7). Fig. 6 shows that, although the activity of the CAT enzyme was increased significantly during different stages of seed development in both fresh and dry seeds, its drying increased compared to those of fresh seeds. However, in the YB stage, it was decreased in both fresh and dry seeds.

Table 2. Effect of organ harvesting type and drying methods at different maturity stages of faba bean on some germination and biochemical characteristics.

Harvesting stage	Organ harvesting type	Drying method	Germination (%)	Soluble proteins content (mg.gr^{-1} fresh weight of embryonic axis)	GR (mmol. gr^{-1} fresh weight of embryonic axis min^{-1})
MS (84 DAS)	Bush	Shade-drying	89^{a-d}	90.66^{bc}	0.048^{h}
		Sun-drying	34^{f}	86.27^{de}	0.023^{ji}
	Pod	Shade-drying	60^{e}	76.70^{h}	0.042^{hi}
		Sun-drying	20e^{g}	73.46^{i}	0.23^{ji}
	Seed	Shade drying	19^{g}	82.19^{f}	0.018^{j}
		Sun-drying	0^{h}	77.24^{h}	0.017^{j}
FS (96 DAS)	Bush	Shade drying	96^{a-c}	83.59^{ef}	0.105^{b-d}
		Sun-drying	85^{cd}	83.03^{f}	0.074^{fg}
	Pod	Shade drying	95^{ab}	83.37^{f}	0.09^{d-f}
		Sun-drying	68^{e}	78.39^{gh}	0.06^{gh}
	Seed	Shade drying	84^{d}	92.84^{b}	0.083^{fe}
		Sun-drying	22^{g}	89.65^{c}	0.02^{j}
GY(108 DAS)	Bush	Shade drying	99^{a}	80.97^{fg}	0.131^{a}
		Sun-drying	94^{a-d}	86.68^{d}	0.113^{a-c}
	Pod	Shade drying	96^{ab}	81.09^{fg}	0.116^{a-c}
		Sun-drying	86^{b-d}	75.60^{hi}	0.122^{ab}
	Seed	Shade drying	96^{ab}	81.85^{f}	0.121^{ab}
		Sun-drying	95^{a-c}	77.73^{h}	0.130^{a}
YB(125 DAS)	Conventional harvest	Conventional drying	89^{abcd}	98.36^{a}	0.099^{c-e}

The mean comparison was conducted by L.S.Means test at the level of 5% probability and columns with common letters are not significantly different at any developmental stages

Glutathione reductase (GR): In all three maturity stages, the activity of GR as same as CAT and SS content was higher in the podded attached and podded detached than depodded one, so that it was higher in the shade-drying than the sun-drying method (Table 2). In MS and FS stages, the pattern of changes in the level of GR activity in the podded attached shade-dried seeds or podded detached shade-dried seeds was similar to that of the CAT compared to depodded shade-dried seeds or depodded sun-dried seeds. However, the activity of this enzyme was maximized at the GY stage, so that the highest activity was observed in the podded attached shade-dried seeds, but it did not differ significantly with other treatments at this stage. In the YB stage, it was decreased similarly to CAT enzyme (Table 2 and Fig. 6). Comparison of GR activity in fresh and dry seeds in different maturity stages showed that although by growing the seed it was increased in both fresh and dry seeds, drying the seed affected it, as its activity was more in dry seeds than fresh seeds (Fig. 8).

Ascorbate peroxidase (APX): Unlike CAT and GR, APX activity was decreasing during seed development, so that the highest amount was observed in MS stage and treatment of the podded attached shade-dried seeds, which had no significant difference only with the podded detached shade-dried seeds in MS stage. In this regard, depodded sun-dried seeds showed the lowest amount. However, in all three stages of MS, FS, and GY, the amount of APX enzyme was not significantly different between the podded attached shade-dried seeds, the podded detached shade-dried seeds, and the depodded sun-dried seeds. The decrease in APX activity continued up to YB stage, but its difference was not significant with GY stage (Fig. 9). Again, although in both dry and fresh seeds the APX activity was decreased significantly during seed maturity, it was higher in dry seeds (Fig. 10).

Soluble proteins content (SP): Results showed that the highest SP content was observed in YB stage (96.3 mg.gr^{-1} fresh weight of embryo axis), which had a significant difference with other treatments. However, despite a higher SP in all three stages of MS, FS, and GY in most of the sun-drying treatments than shade-drying treatments, the accumulation of these proteins in almost all treatments was approximately close (Table 2). SP content did not show any significant changes in both fresh and dry seeds. However, at the first harvesting stage, this amount was higher in the dry seeds than fresh ones, but higher in fresh seeds than that of dried seeds thereafter (Fig. 11).

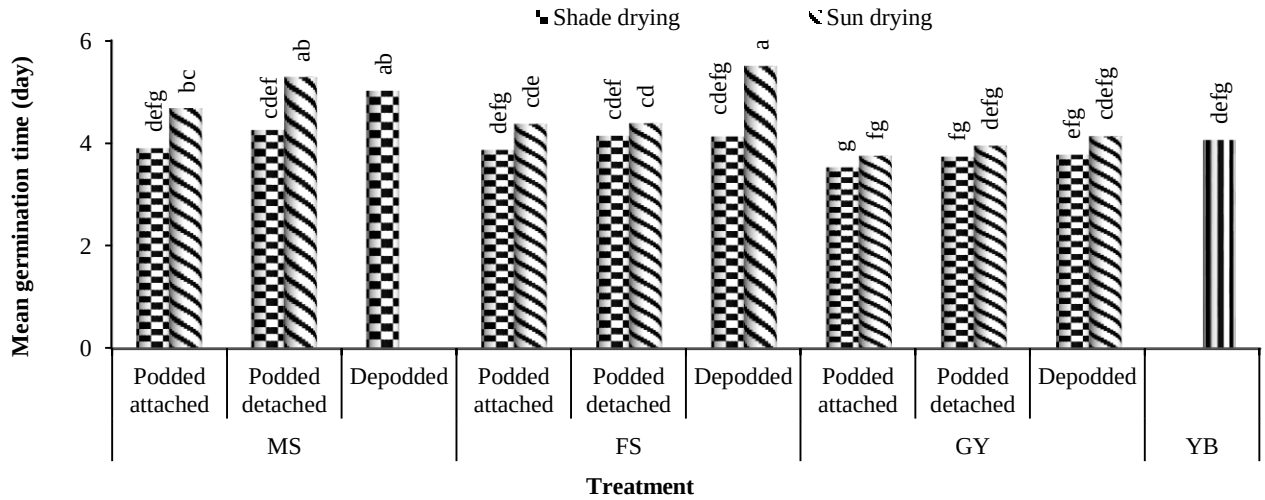

Fig. 3. Mean comparison of organ harvesting type and drying methods at different maturity stages of faba bean for MGT. Each data is average of five replications. Mean comparison was conducted by LSD test at and bars with common letters are not significantly different 5% statistical level.

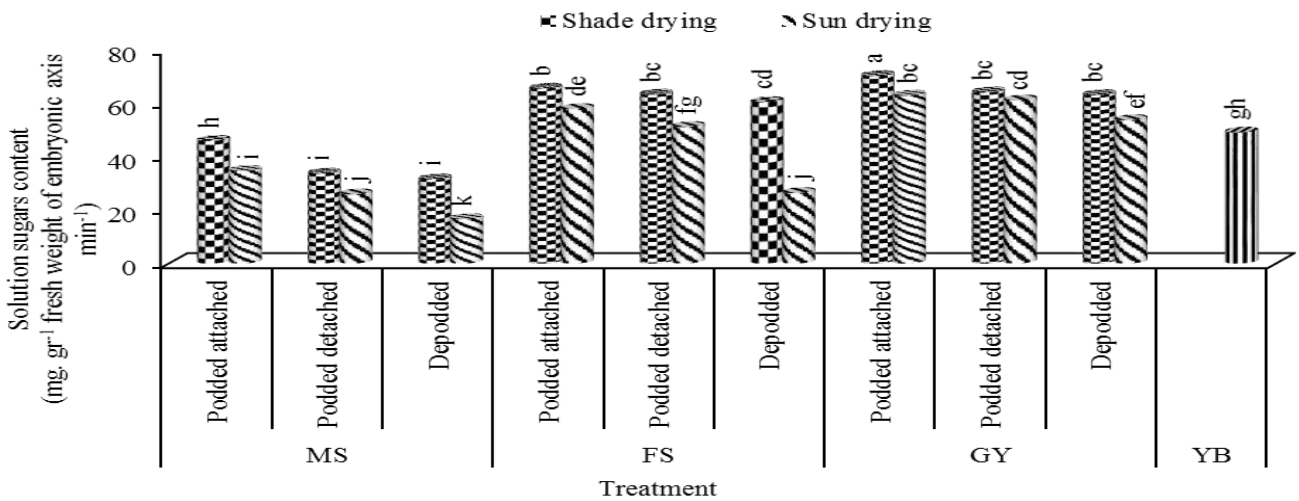

Fig. 4. Effect of organ harvesting type and drying methods at different maturity stages of faba bean on SS content (%). Each data is average of five replications. Mean comparison was conducted by LSD test at and bars with common letters are not significantly different 5% statistical level.

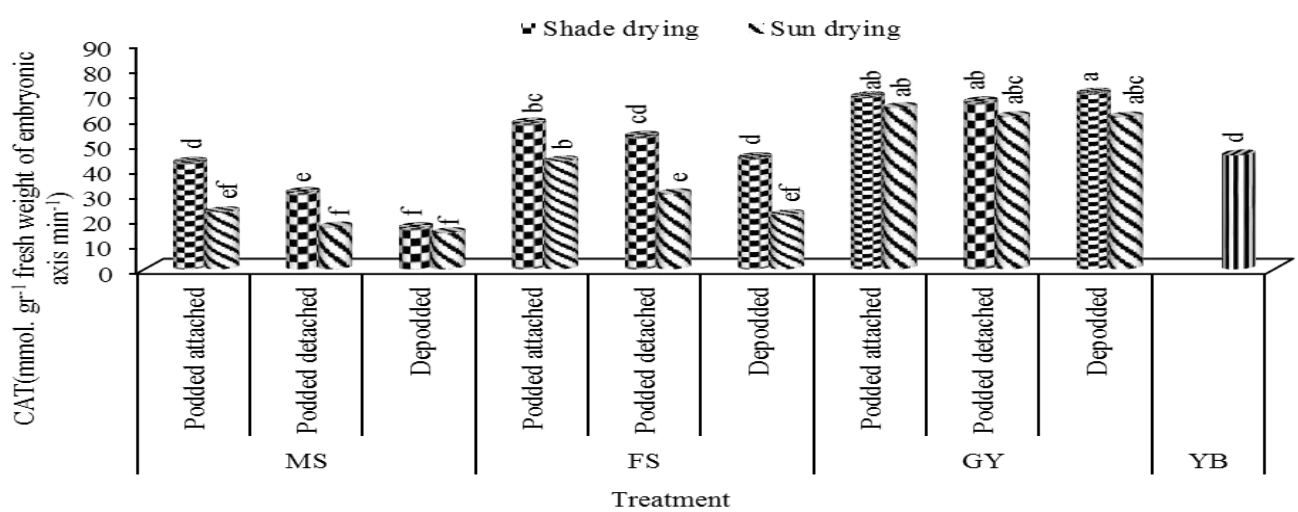

Fig. 6. Effect of organ harvesting type and drying methods at different maturity stages of faba bean on CAT enzyme activity. Each data is average of five replications. Mean comparison was conducted by LSD test at and bars with common letters are not significantly different 5% statistical level.

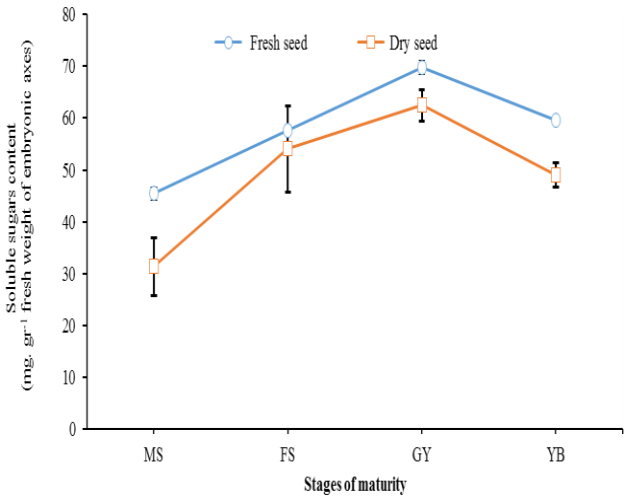

Fig. 5. Change in the SS content of fresh and dried seeds of faba bean seeds during different maturity stages. Vertical lines on the points indicate the standard error.

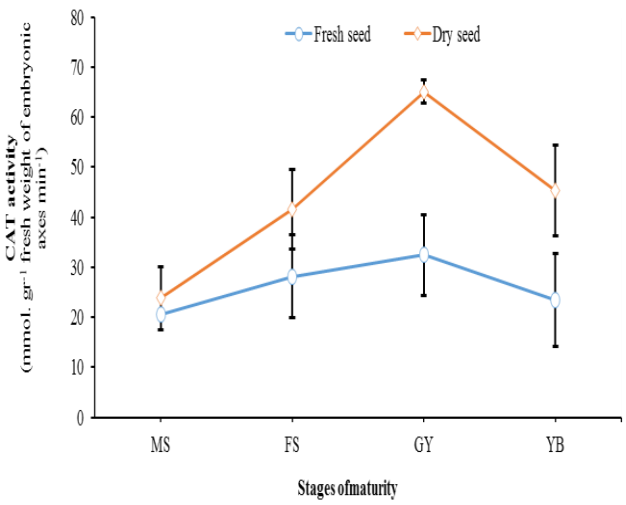

Fig. 7. Changes in the CAT activity of fresh and dried seeds of faba bean seeds during of faba bean seeds during different maturity stages. Vertical lines on the points indicate the standard error.

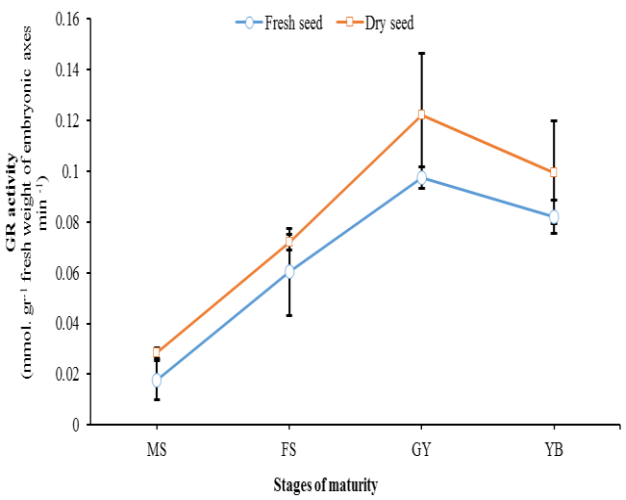

Fig. 8. Changes in the GR activity of fresh and dried seeds of faba bean seeds during different maturity stages. Vertical lines on the points indicate the standard error.

Discussion

The results of the present study showed that acquisition of desiccation tolerance that prevailed, based on germinability and biochemical parameters were significantly affected by maturity stage, the harvested organ type, and the drying method. For example, GP, NS (as a sign of acquisition of desiccation tolerance), SS content, and the activity of CAT and GR enzymes were increased during seed maturity, and at each stage, it was higher in shade-dried seeds compared to those of the sun-dried seeds. Also, it was higher in the podded attached dried seeds and podded detached dried seeds than the depodded dried seeds (Table 2 and Figs. 2, 4, 6, and 9). Seed germination capacities can be affected by harvesting time (seed maturity stage) and drying method (Ellis *et al.*, 1987). It has also been reported in some legumes (Samarah, 2005 and 2006; Samarah *et al.*, 2009; Ennen, 2011; Corbineau *et al.*, 2000) and cereals (Samarah *et al.*, 2010) that the type of organ harvested and the seed dried inside it or separated from it can also affect the germination capabilities and desiccation tolerance.

In this research, it was observed that faba bean seeds reached the maximum dry weight at the GY stage (Fig. 1). At this stage, the moisture content of seed was about 47.62%, which was close to the results obtained for soybean (Samarah *et al.*, 2009), bean (Bailly *et al.*, 2001), common vetch (Samara, 2006), and cowpea (Eskandari, 2012). The determination of the physiological maturity stage in the seed using the number of days after flowering (DAF) was found to be an inaccurate method due to the effects of different factors including environmental conditions on the seed growth and maturity rate. Seed moisture content can be used to standardize harvesting time in studies of seed development and maturity and, thus, it is a better indicator of the physiological maturity of seeds (Miles *et al.*, 1988).

Our results showed that developmental stage in which the faba bean seeds reached the maximum germination (acquisition of desiccation tolerance) depends on the drying method and the type of organ harvested (Table 2). As can be seen from Table 2, the podded attached and podded detached compared to the depodded methods as well as the shade-drying method compared to the sun-drying method are more efficient methods in terms of the seed quality. Results of Samarah (2005 &2006) on common vetch showed that the highest germination indices were related to dried seeds in the podded attached and podded detached, while the lowest ones were related to the seeds isolated from the pods (depodded), especially in the early stages of seed maturity. Improving seed germination capacities in these conditions may be due to the slow rate of drying or gaining a higher dry weight. In fact, these seeds may continue to maturate during drying of the pods, leading to protection changes related to the desiccation tolerance during drying of the seed. This increase may also be due to allowing the seed to be continuously developed within the pod (Fu *et al.*, 1997).

Samarah *et al.,* (2009 & 2010) reported that a slow drying of wheat seeds in the spike and soybeans in the pod, significantly increased the germination capacities. However, the damage caused by ultraviolet radiation to essential structures of the seed, especially embryonic cells in sun-drying method (Doijode, 1990), is also effective in reducing the germinability of sun-drying treatments, especially in the harvested organ type of depoded, as well as immature seeds. Increasing germination during seed development (Table 2) may be due to an increase in seed's food reserves (Alizadeh Benab *et al.,* 2006), which has been reported in soybean (Ennen, 2011), pea (Ghasemi Golazani *et al.,* 2011b), and corn (Dayal *et al.,* 2015).

The desiccation tolerance in faba bean seeds was evaluated through evaluating the ability of germination and seedling production after drying. Like other species such as lupine (Garnczarska *et al.,* 2009), bean (Bailly *et al.,* 2001), soybeans (Blackman *et al.,* 1992), peas (Corbineau *et al.,* 2000), and lupine (Gorecki *et al.,* 1997), the acquisition of desiccation tolerance (germinability) in faba bean seeds began in the early stages of maturity (in this research in the MS stage) and reached the maximum at the end of the accumulation of dry matter, i.e., GY stage (Table 2 and Fig. 2). However, the acquisition of desiccation tolerance depended on the rate of drying, harvesting organ type, and drying method. In some legumes, it has also been reported that the acquisition of desiccation tolerance depends on the desiccation condition including the rate of water loss from the seed (Blackman *et al.,* 1992, Sanhewe and Ellis, 1996; Black *et al.,* 1999; Corbineau *et al.,* 2000). As can be seen from germination characteristics in Table 2 and Fig. 2, the seeds were harvested in the form of the podded attached to the plant and podded detached and shade-drying resulted in a higher percentage of seeds tolerant to the desiccation. Even seeds harvested in the form of pods attached to the plants or pods detached that dried in the sun, had a higher germination than seeds isolated from the pods dried in the shade (MS and FS stages). Drying seeds of wheat in the spike (Samarah *et al.,* 2010) and seeds of pea (Corbineau *et al.,* 2000) in the pod also improved the desiccation tolerance.

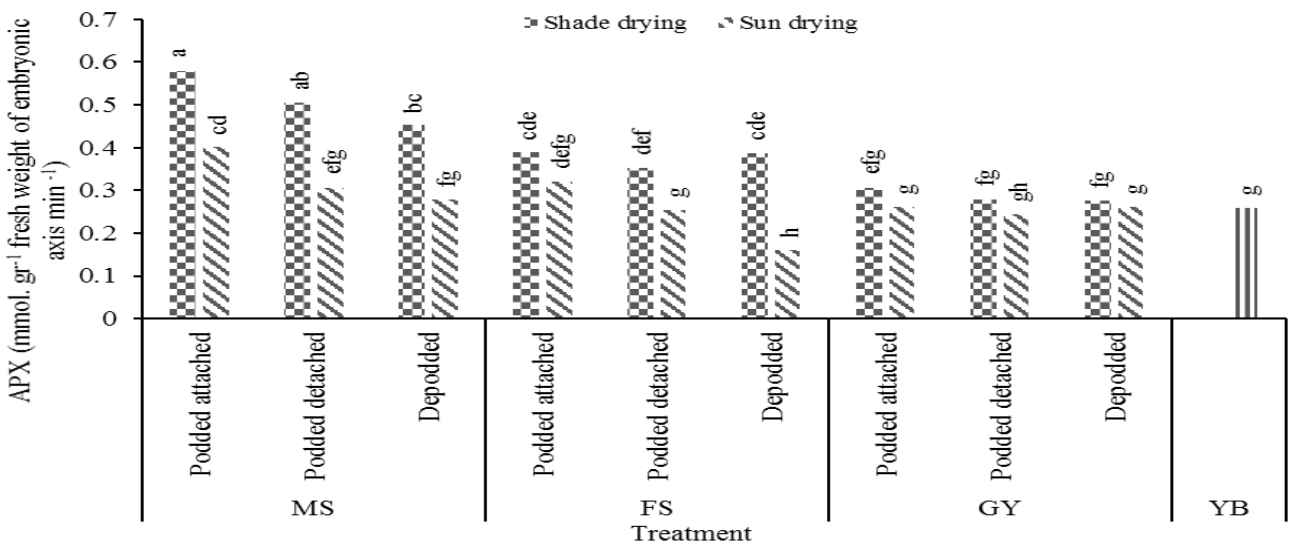

Fig. 9. Effect of organ harvesting type and drying methods at different maturity stages of faba bean on APX enzyme activity. Each data is average of five replications. Mean comparison was conducted by LSD test at and bars with common letters are not significantly different 5% statistical level.

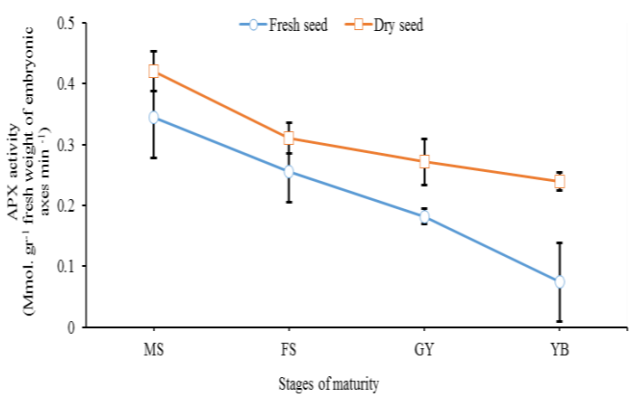

Fig. 10. Changes in the APX activity of fresh and dried seeds of faba bean seeds during of faba bean seeds during different maturity stages. Vertical lines on the points indicate the standard error.

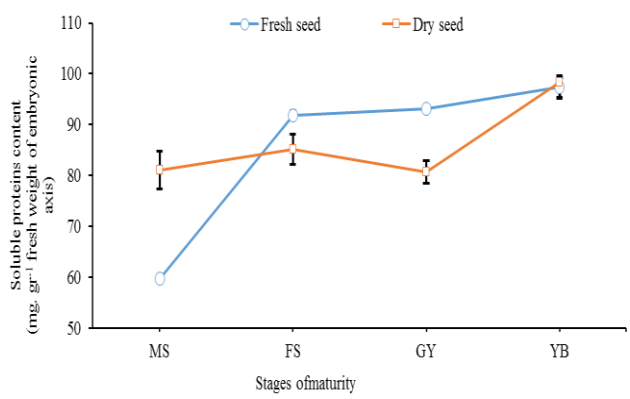

Fig. 11. Changes in the SP content of fresh and dried seeds of faba bean seeds during of faba bean seeds during different maturity stages. Vertical lines on the points indicate the standard error.

The acquisition of desiccation tolerance in faba bean seeds was along with the accumulation of soluble sugars in the embryonic axis (germination characteristics and Fig. 4). Such relations were found previously between the increase in SS (oligosaccharides) and acquisition of desiccation tolerance in wheat (Lehner *et al.,* 2006; Lehner *et al.,* 2008), bean (Bailly *et al.,* 2001), and pea (Corbineau *et al.,* 2000). The slow drying of seeds results in the accumulation of more SS and, as a result, a further development of tolerance to desiccation. Verdier *et al.,* (2013) also reported that the acquisition of desiccation tolerance in *Medicago truncatula* was associated with the response of genes related with LEA proteins, heat shock proteins, and increasing the content of SS, including stachyose. According to Angelovici *et al.,* (2010), the accumulation of SS such as sucrose, raffinose, galactinol, and trehalose is also associated with the acquisition of desiccation tolerance. It has been reported that the biosynthesis of oligosaccharides associated with its desiccation tolerance (sucrose, raffinose, galactinol, and stachyose verbascose) is regulated by the rate of water loss from the seed. In this regard, a slower rate increases the amount of oligosaccharides synthesized in the embryonic axis of the seed, leading to an increase in the desiccation tolerance and finally germination capacity and seed vigor. The accumulation of these sugars is probably due to an increase in the activity of the enzymes of galactinol synthase, raffinose synthase, and stachyose synthase, which are preferably occurred at a slow loss of water from the seeds (Corbiniuo *et al.,* 2000). In this experiment, the process of changes in germination indices (acquisition of desiccation tolerance) with SS in different treatments during seed development was almost the same. The positive and significant correlation between SS content and germination indices ($r = +0.94$ **, results have not been shown) also confirms the role of soluble sugars in enhancing germination. The higher SS in fresh seeds than dry seeds (Fig. 5) may be due to differences in the drying rate of the seed or type of accumulated sugar (Lehner *et al.,* 2006).

The desiccation of plant tissues increases the production of reactive oxygen species (AOS) that can react together and cause many oxidative degradation processes (Smirnoff, 1993). In this situation, the antioxidant defense system plays an important role in acquiring the desiccation tolerance (Hendry *et al.,* 1993). It seems that achieving the desiccation tolerance in faba bean seeds is associated with the activation of the antioxidant defense system. Treatments that showed germination and high levels of soluble sugars (for example, podded attached, and podded detached under shade and sun conditions, especially in the MS and FS stages) had simultaneously the high activity of GR and CAT and the low activity of APX (Table 2 and Figs. 6 and 9). However, this trend was inverse in seeds without germination or low germination. Contrary to APX, in both dry and fresh seeds the GR and CAT activities were increased during seed growth, but it was more in dry seeds compared to fresh seeds (Figs. 7, 8, and 10). These results show that the desiccation tolerance was along with the increase in GR and CAT activity, and in the presence of soluble sugars. The changes in APX activity (Figs. 9 and 10) as well as the negative and non-significant correlation (the results have not been shown) with germination indices reveal that although the amount of this enzyme was high at the early stages of maturity (MS stage), but it seems that it has no considerable role in acquisition of desiccation tolerance. In lupine (Garnczarska *et al.,* 2009), bean (Bailly *et al.,* 2001) and corn (Huang & Sung, 2013), it has been reported that the acquisition of desiccation tolerance initiated with an increase in the activity of CAT and GR enzymes and reached the maximum amount at the maximum activity of these enzymes, while the APX enzyme showed an opposite trend contrasting to these two enzymes and did not play a role in achieving tolerance to desiccation. It was also reported that in corn (Huang & Sung, 2013), the activity of these enzymes in dried seeds was higher than that of fresh seeds. This result is consistent with that of our research concerning the activity of these enzymes in dry and fresh seeds (Figs. 7, 8, and 10). Similar results have been reported in beans (Bailly *et al.,* 2001) and sunflowers (Bailly *et al.,* 2004).

Maintaining the enzymatic antioxidant defense system in relation to the acquisition of desiccation tolerance might lead to the modulation of the antioxidant or the ROS levels (Garnczarska *et al.,* 2009). CAT has shown a variety of isoforms pattern during the development of cotton and corn seeds (Scandalios, 1997). On the other hand, since APX plays a role in cell division, its activity decreased during development and maturity (Arrigoni *et al.,* 1992) (Fig. 10). Glutathione and GR, in addition to antioxidant properties, play a role in various metabolic processes such as regulating the activity of enzymes, regulating gene expression, and molecular signaling (Mullineaux & Creissen, 1997). Changes in the activity of these enzymes during seed growth can also be related to changes in organelles activity or their differentiation during the desiccation (Vertucci & Farrant, 1995). Finally, it should be noted that these enzymes belong to cellular compartments such as mitochondria (CAT and GR), cytosol (APX and GR), plastid (GR), and peroxisomes (CAT) (Scandalios, 1997). Since different levels of moisture are necessary to stimulate cellular events, seed drying speed can have a different effect on the critical moisture content in these compartments (Vertucci & Farant, 1995).

Delay in harvesting may reduce germination indices due to the onset of aging processes in seeds on the mother plant (Gurusamy & Thiagarajan, 1998). Besides, accumulation of AOS during the aging process can cause deactivation of enzymes and, consequently, the loss of their activity. Therefore, the decrease in the activity of enzymes and the SS content, and the increase in SP content at the YB stage may be because of the onset of aging processes due to the delayed harvest as well as the common drying (sun-drying in the field).

The results of carbohydrate accumulation and antioxidant enzymes activity are approximately similar to those of germination indices so that a positive and significant correlation was observed between these

indices and germination (results have not been shown), suggesting the role of these compounds in the acquisition of desiccation tolerance for the faba bean seeds. However, the accumulation of SP was almost the same in both desiccation tolerant and intolerant seeds (without and with the germinability), implying that the presence of these proteins is necessary to achieve tolerance to desiccation, but they are not enough on their own. Nevertheless, this subject requires more research.

Conclusion

Generally, the results of the present work indicated that seed dry weight, SS content, CAT, and GR enzymes activity, GP, and NS increased during seed growth until GY stage and decreased during YB stage (unlike MGT). As a result, the maximum and minimum desiccation tolerances (seed quality) were obtained at the GY and YB stages, respectively. Hence, Harington's hypothesis is confirmed in our research. Also, the quality of seed in shade-drying was higher compared to that of sun-drying. In the harvesting, organ type of the podded attached to plant was more than the podded attached while in the podded attached it was more than that in the depodded seeds. Therefore, the optimum harvesting stage, harvested organ type, and drying method of faba bean seeds in this research were in GY stage, the podded attached to plant, and shade-drying, respectively. The SS content of seed and the activity of CAT and GR enzymes may have a role in the development of the desiccation tolerance and enhancement of germination capabilities of faba bean seeds. Therefore, they can be proposed as the assessment indicators for the biochemical quality of the harvested seeds.

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