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Mobilization of seed reserves during germination and early seedling growth of two sunflower cultivars

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Summary

The present study was carried out to determine the mobilization of seed storage components of sunflower seeds during germination and early seedling development. Two sunflower cultivars (DUET CL and TR 3080) were used as plant materials. Seeds were germinated for 120 h and samples were taken every 24 h. The total chlorophyll and carotenoid contents of the germinating seeds of both cultivars significantly increased till 96 h, and then decreased. While the total soluble and reducing sugar contents were decreased during the first 24 h, their amounts increased significantly afterwards. The total protein contents of the germinating seeds of TR 3080 and DUET CL decreased from 48.1 % and 40.9 % to 35.5 % and 28.4 %, respectively. However, their free amino acid contents were steadily increased during germination and early seedling growth. The oil contents of DUET CL and TR 3080 started to decrease significantly after 72 h and dropped to 41.3 % and 40.2 %, respectively, at the end of the study. Free fatty acid contents of the seeds increased until 72 h, but decreased thereafter. While, oleic acid contents of the cultivars decreased during the germination period, their linoleic acid contents increased. These results suggest that significant changes occur in the pigment, sugar, lipid and protein metabolisms during germination and early seedling growth period of sunflower.

Introduction

Sunflower (*Helianthus annuus* L.) belongs to Asteraceae family and is one of the significant oil seed crops in the world. Sunflower seeds contain 35-50 % oil, 20-30 % protein, 20-40 % carbohydrate, 20-30 % husk and 4-6 % ash (Weiss, 2000). Sunflower oil is rich in unsaturated fatty acids (80-90 %). Commercial sunflower oil containes 5 fatty acids including palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$). Recently, the development of sunflower cultivars with high oleic acid content (<85 %) further increased its industrial use (Weiss, 2000). Moroever, the remaining meal after the removal of oil from seeds is rich in tryptophan and it is important for animal feed (Balasaraswatti and Sadasivam, 1997).

In general, there are two substantial changes take place in the plants after pollination and fertilization: One is the increase in seed volume and the other is the change in biochemical and physiological characteristics. The former involves the division, expansion and differentiation of seed cells and the latter involves the changes in seed metabolites such as storage lipids, proteins, carbonhydrates and other seed storage metabolic substances (Satyanarayana et al., 2011). Sucrose synthesized during maturation of oil seeds is the source of carbon for triacylglycerol (TAG) synthesis (Bewley and Black, 1994).

Macromolecules accumulated in seeds are used as an energy source for early seedling development and seed germination. Germination begins with water uptake by the seed (imbibition) and the emergence of embryonic axis, usually the radicle, through the structures surrounding it (Bewley et al., 2013). When seed germination begins, starch and proteins are converted to sugars and amino acids within the starch granules and protein storage vacuoles, by diastase and protease enzymes, respectively (Wilson, 2006). TAGs are hydrolysed by lipases, enzymes catalyzing the hydrolytic cleavage of the fatty acid ester bonds, to yield glycerol and free fatty acids (Theimer and Rosnitschek, 1978). Free fatty acids enter the glyoxysome for conversion to oxaloacetic acid (OAA), passes into the mitochondrion, and ultimately into the cytosol for conversion to sucrose, which then transported as an energy source from cotyledons to the growing axis of seedling (Graham, 2008).

Almost all TAGs present in oleiferous seeds are lost during seed germination and seedling development (Muto and Beevers, 1974; Yaniv et al., 1998; Rabiei et al., 2007; Kim et al., 2011; Tonguç et al., 2012). Although there are studies regarding the physiology and mobilization of sunflower seed oil during germination (Balasaraswatti and Sadasivam, 1997; Rosales et al., 1998; Moya et al., 2000; Munshi et al., 2007; Rabiei et al., 2007), little is known about the changes in other seed reserves during the germination. The objective of the present study was to investigate the mobilization of seed reserve molecules during the germination and early seedling growth of sunflower.

Materials and methods

Two sunflower cultivars, TR 3080 and DUET CL were used as plant materials. TR 3080 is a high linoleic acid and DUET CL is a high oleic acid cultivar. Seeds were surface sterilized with 2 % sodium hypochlorite solution for 5 min, and then washed with distilled water several times to remove residual sodium hypochlorite. Seeds were germinated in 9 cm petri dishes on 2 sheets of filter paper moistened with distilled water. Petri dishes were incubated at 25 °C under alternating light and dark periods (12 h light/dark periods). 25 seeds were placed in each petri dish, and 3 replications were used for the analysis of each parameter. Seeds were germinated for 120 h, and the germinated seeds were removed from the incubator at every 24 h for analysis. The cotyledons from germinating seeds and growing seedlings were separated and dried for at least 24 h at 50 °C or until they reached to a constant weight (Tonguç et al., 2012). The germination percentage of DUET CL and TR 3080 were 96.2 and 98.5 %, respectively. Non-germinated or unevenly germinated seeds were discarded. For all analysis, the seeds were dehulled, and only the grinded dried cotyledons were used. The hull content of both cultivars was 23.5 %. The total protein content was determined using the defatted and dehulled seeds. Total chlorophyll and total carotenoid contents were determined from fresh cotyledons.

The dry matter content of fresh cotyledons (2 g) was determined gravimetrically, by drying to a constant weight at 105 °C [Dry matter (%) = dry weight / fresh weight × 100] (AOAC, 1990). Ash content of samples was determined following the method of AOAC (1990).

Total chlorophyll content of fresh cotyledons (0.25 g) was deter-

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mined according to KIRK and ALLEN (1965) with the following equation: Total chlorophyll (mg g^{-1}) = [(0.0202 × A_{645}) + (0.00802 × A_{663}) × 10] / sample weight. For total carotenoids content of fresh cotyledons, 10 ml of acetone-hexane (4:6) solvent was added on 0.25 g sample and the mixture was homogenized. Two phases were separated, and the aliquots were taken from the upper phase and read on a spectrophotometer (PG Instruments) at 480, 645 and 663 nm wavelengths. Total carotenoid content was calculated according to MILLER et al. (1993) using the following equation: Total carotenoids (mg g^{-1}) = A_{480} + (0.114 × A_{663} – 0.638 × A_{645}).

Total soluble and reducing sugars were extracted as described (TONGUÇ et al., 2012). The total soluble sugar content was determined by the phenol sulfuric acid assay (DUBOIS et al., 1956) and the reducing sugar content was determined following the SOMOGYI (1952) method. Glucose was used as standard and the results were expressed as mg g^{-1} dry weight.

The protein extraction was performed according to the method of LARSON and BEEVERS (1965) and the total protein content was determined as described (HARTREE and LOWRY, 1995). Bovine serum albumin was used as standard. Amino acids were extracted according to NOCTOR et al. (2007). Total free amino acid content was determined with the ninhydrine method as described by LEE and TAKAHASHI (1966). Standard curve was prepared using L-valine as standard.

The oil content and fatty acid composition were determined using Nuclear magnetic resonance (NMR, Brükermq$_{one}$) and Gas chromatography (GC, Perkin Elmer Auto System XL), respectively. The oil samples (50-100 mg) were obtained with cold extraction and converted to its fatty acid methyl esters (FAME) as described by MARQUARD (1987). The conditions for GC analysis were as follows: capillary column, MN FFAP (50 m × 0.32 mm i.d., film thickness, 0.25 m), oven temperature kept at 120 °C for 1 min and programmed to 250 °C at a rate of 6 °C min^{-1}, and then constant at 240 °C for 15 min, total run time 60 min, injector temperature 250 °C, detector (70 eV) temperature 260 °C, flow rate for helium 40 ml min^{-1}, split ratio 1/20 ml min^{-1}, injection volume 0.5 µl.

The free fatty acid content of lipids was determined colorimetrically according to the method of LOWRY and TINSLEY (1976). Samples (1 g) were extracted with hexane and the extracted lipids (4 µl) were dissolved in benzene (5 ml). Cupric acetate-pyridine reagent (1 ml) was added and the samples were vortexed for 90 s. The mixture was then centrifuged for 3 min at 1500 g. 3 mls of supernatant were used to determine the free fatty acid contents of the samples. The total free fatty acid content of samples (%) was determined as oleic acid equivalents.

The experiment was set up according to a completely randomized experimental design with 3 replications. The data were subjected to the analysis of variance (ANOVA) using SAS (2009) program (INC SAS/STAT user's guide release 7.0, Cary, NC, USA). Differences among means were determined using Least Significant Difference (LSD) test.

Results and discussion

Plants use seed coat to protect embryo from the environmental effects until seeds germinate and seed coats are not used as seed storage compartment. Therefore; seed coats of the sunflower cultivars were removed prior to analysis. Mobilization of the main reserves such as starch, oil and protein in seed storage tissues occurs following the completion of germination to supply nutrients for the growing seedling until it develops true leaves. Reserve mobilization differs among plant species during germination depending on the accumulated reserves (BEWLEY et al., 2013). In the present study, breakdown and mobilization of different molecules were investigated. The germination period, cultivar and the germination period × cultivar interaction were significant ($P<0.01$) for dry matter, total chlorophyll, total soluble and reducing sugar contents during the study period (Tab. 1). While the germination period and cultivar effects were significant ($P<0.01$) for ash, total protein and free fatty acid contents, the germination period × cultivar interaction was not significant for these parameters. For free amino acid and oil contents, the germination period and the gemination period × cultivar interaction were significant at 1%, and the cultivar effect was significant at the 5% level of significance. For the total carotenoid content of the cotyledones during the germination, the germination period and the cultivar × germination period interaction were significant ($P<0.05$), but the cultivar effect was not significant (Tab. 1).

The dry matter contents of both cultivars decreased gradually throughout the germination period. At 0 h, the dry matter contents of TR 3080 and DUET CL were 95.2% and 95.7%, respectively. At 120 h, the dry matter contents decreased to 36.8% and 27.3%, respectively. The highest dry matter loss in the cotyledons was observed at the end of the first 24 h period (Fig. 1a). The dry matter loss in germinating seeds depends on the germination period. A negative correlation was reported between the dry matter loss and

Tab. 1: Analysis of variance (ANOVA) results for the examined parameters during germination and early seedling growth in sunflower cotyledons (F values).

Sources of Variation	**DM (mg g^{-1})**	**AC (%)**	**TCC (mg 100 g^{-1})**	**TC (mg 100 g^{-1})**	**TSS (mg g^{-1})**
Germination period	764.5**	6.12**	617.2**	258.8*	564.9**
Cultivar	123.6**	22.7**	37.9**	2.8	180.4**
Germination period × cultivar	7.63**	2.15	6.6**	2.7*	61.2**
Coefficient of variation (%)	3.4	6.8	4.8	7.4	5.4
	RS (mg g^{-1})	**PC (%)**	**FAA (%)**	**OC (%)**	**FFA (%)**
Germination period	1478.8**	47.6**	225.7**	235.3**	16.5**
Cultivar	139.6**	157.1**	5.65*	4.8*	108.2**
Germination period × cultivar	164.1**	1.7	4.5**	5.1**	0.5
Coefficient of variation (%)	4.7	5.0	10.5	1.6	10.7

*: $p<0.05$, **: $p<0.01$

DM (mg g^{-1}): Dry matter, AC (%): Ash content, TCC (mg 100 g^{-1}): Total chlorophyll content, TC (mg 100 g^{-1}): Total carotenoid content, TSS (mg g^{-1}): Total soluble sugar, RS (mg g^{-1}): Reduced sugar, PC (%): Total protein content, FAA (%): Free amino acid content, OC (%): oil content, FFA (%): Free fatty acid content.

seed germination period, due to the water uptake and excessive respiration process in the germinating seeds (Tarasevičienė et al., 2009). The dry matter loss with water uptake throughout the germination period was reported in seeds of different species including linseed (Wanasundara et al., 1999), cotton (Joshi and Doctor, 1975) and tobacco (Koiwai and Matsuzaki, 1990).

The ash contents of the cultivars fluctuated during the germination period (Fig. 1b). At 0 h, DUET CL and TR 3080 had 4.99% and 4.19% ash contents, while their ash contents increased to 5.54% and 4.88%, respectively, at 24 h, thereafter decreased till 72 h but the ash content of the cultivars increased again during the last 48 h period of the study. The ash contents of the sunflower cultivars also increased during the germination period. Increased ash content might be related to the increase in the relative proportion of minerals due to the loss of other seed storage reserves (Borek et al., 2006). Similar results were reported in amaranth (Colmenares De Ruiz and Bressani, 1990) and sorghum seeds (Elmaiki et al., 1999). However, in contrast to our findings, Taraseviciene et al. (2009) reported that the ash content of broccoli seed remained constant during the germination.

At the beginning of germination, DUET CL had a total chlorophyll content of 7.4 mg 100 g^{-1} and TR 3080 had 13.9 mg 100 g^{-1}. Total chlorophyll contents of the cotyledons of both cultivars increased from 0 to 96 h, but decreased thereafter. At the end of the study period, the total chlorophyll contents were 187.9 and 199.2 mg 100 g^{-1} for TR3080 and DUET CL, respectively (Fig. 1c). The changes in total carotenoid contents of the cultivars were similar to the changes in total chlorophyll contents during the germination period. Chlorophyll synthesis in plants ceases after the emergence of leaves and depletion of reserves (Harris et al., 1986). Total chlorophyll contents of both cultivars increased till 96 h but thereafter their chlorophyll contents decreased when the first true leaves of seedlings developed. As observed in the present study, Bush and Grunwald (1972) reported an increase during the first 6 days of the germination but thereafter a decrease in the chlorophyll content of tobacco seeds.

TR 3080 and DUET CL had total carotenoid contents of 4.2 µg 100 g^{-1} and 1.5 µg 100 g^{-1} respectively, at 0 h. Similar to the changes in the chlorophyll content, the total carotenoid contents of cotyledons increased up to 96 h and reached to their highest levels (52.3 µg 100 g^{-1} for TR 3080 and 47.6 µg 100 g^{-1} for DUET CL), but thereafter they started to decrease (Fig. 1d). Chlorophyll is sythesized in cotyledons with the start of germination in dicot seeds. Carotenoids are essential components of the photosynthetic machinery in plants and play significant roles in during germination and the restriction of free radical induced membrane deterioration and seed ageing (Calucci et al., 2004). The synthesis of carotenoids in seeds starts with the germination of seeds under light conditions (Von Lintig et al., 1997). Similar results were also observed during the germination of tobacco seeds (Bush and Grunwald, 1972).

The total soluble and reducing sugar contents of both cultivars showed similar trends during the study period (Fig. 1e, f). The total soluble and reducing sugar contents of the cultivars were 7.3 mg g^{-1} and 1.8 mg g^{-1} for TR3080 and 11.0 mg g^{-1} and 1.5 mg g^{-1}, for DUET CL respectively, at 0 h. The total soluble and reducing sugar contents of the cultivars decreased during the first 24 h, and thereafter both total soluble and reducing sugars contents increased. The total soluble and reducing sugar contents of the cultivars at the 120 h was determined to be 28.6 mg g^{-1} and 6.4 mg g^{-1} for TR3080 and 44.4 mg g^{-1} and 10.4 mg g^{-1} for DUET CL, respectively. The carbonhydrate source used throughout the germination period in sunflower seed is glucose (Nascimento Do et al., 1994). At the first 24 h of germination, total soluble and reducing sugars contents decreased as they are the first reserves to be mobilized during the early germination period. Sugars generally used as the initial energy sources and supplied with the hydrolyses of complex carbonhydrates and the synthesis from triglycerides in oilseed crops at the beginning of germination (Tonguç et al., 2012). The free fatty acid contents of both cultivars increased slightly at the first 24 h (Fig. 1j). After 24 h germination period, TSS and RS contents significantly increased indicating an accelerated conversion of reserves to sugars along with the development of cotyledeons with chlorophyll synthesis for starting photosynthesis.

The total protein content of TR 3080 (48.1%) was higher than that of DUET CL (40.9%). The total protein contents of both cultivars decreased throughout the study period. The total protein content of TR 3080 showed a high rate of drop between 24 and 72 h, but the protein content of the DUET CL demonstrated a high rate of decrease between 0 and 48 h. At the end of 120 h, the higher protein degregation was observed in DUET CL (30.6%) as compared to the TR 3080 which showed a 26.2% reduction with respect to the total protein content at 0 h (Fig. 1g). The free amino acid contents of both cultivars steadily increased during the study period. The free amino acid content was 0.59% for TR 3080 at 0 h, and increased to 5.07% at 120 h. The free amino acid content of DUET CL increased from 0.28% to 5.62% during the same period. At the first 24 h, TR 3080 had higher free amino acid content as compared to DUET CL, but the free amino acid content of DUET CL was higher at the other time periods (Fig. 1h). Starch, hemicellulose, or TAGs in cotyledons are the alternative sugar sources for the growing axis during the germination, but amino acids are not used to produce sugars (Balasaraswathi and Sadasivam, 1997). Free amino acid contents of the seeds were very low at the beginning of the study. Storage proteins are proteolysed by different enzymes in different parts of the seed with imbibition and studied comprehensively in vetch and buckwheat seeds (Bewley et al., 2013). The total protein content of cotyledons dramatically decreased at the 24 and 48 h, and thereafter continued to decrease at a slower pace during the study period. At the same time, the free amino acid contents of cultivars increased significantly indicating the start of storage protein mobilization to produce free amino acids for the syntheses of protein components of the growing cells. The amino acids used for protein synthesis and assembly are obtained from the reserves in the cotyledons (Bewley et al., 2013). Balasaraswathi and Sadasivam (1997) reported that globulins and albumins were the storage proteins used in the initial period of germination in sunflower. The reductions in total protein contents during the germination and initial seedling development have been reported in different species including *Sterculia urens* (Satyanarayana et al., 2011), soybean (Kim et al., 2011) and safflower (Tonguç et al., 2012).

The oil contents of both cultivars did not show a significant change during the 48 h germination period, but their contents started to decrease after 48 h. The highest reduction in oil content was observed after 72 h for both cultivars. The oil contents of the cultivars decreased from 52.4% and 51.7% to 40.2% and 41.3% for TR3080 and DUET CL, respectively (Fig. 1i). The free fatty acids contents of extracted oils from the developing cotyledons were 0.71% for TR 3080 and 0.49% for DUET CL at the beginning of the study. Their contents increased to 1.10% and 0.80%, at 72 h, respectively. Then, the free fatty acids content of TR 3080 decreased to 0.84%, at 120 h. The free fatty acid content of DUET CL did not change between 72 and 96 h, but afterwards it decreased to 0.57% (Fig. 1j). The oil contents of the cultivars slightly increased at the beginning of the study but thereafter their oil contents decreased throughout the germination and seedling development. The most dramatic decrease in oil content was observed after 72 and 96 h. It was reported that oils were not used as an energy source till 1-3 days of germinating seeds of many plant species including safflower (Tonguç et al., 2012), castor bean (Muto and Beevers, 1974), sinapis and crambe (Yaniv et al., 1998) and were hydrolyzed later on during the germination

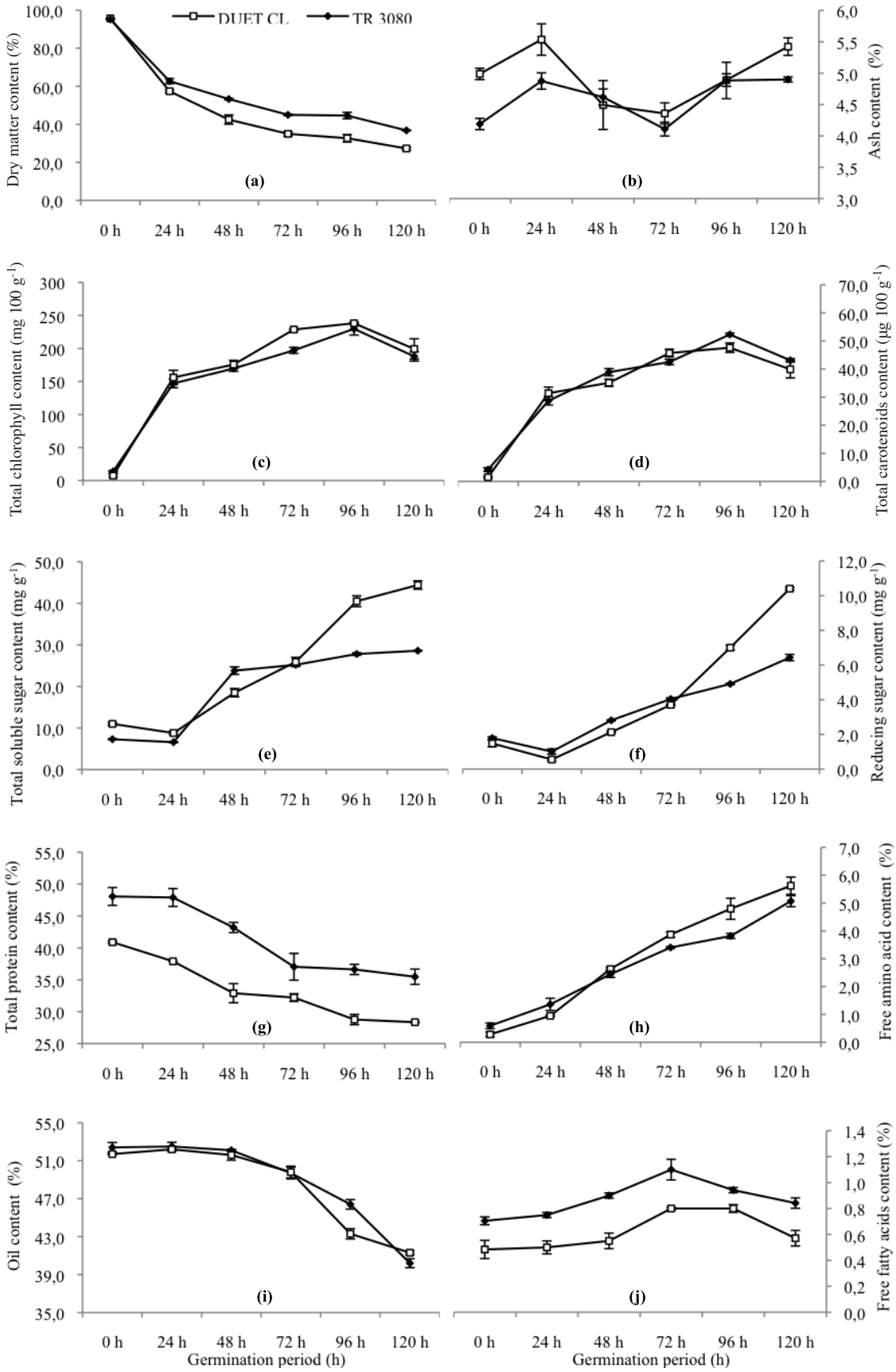

Fig. 1: Change in dry matter content (a), ash content (b), total chlorophyll content (c), total carotenoid content (d), total soluble sugar content (e), reduced sugar content (f), total protein content (g), free amino acid content (h), oil content (i) and free fatty acid content (j) of cultivars during germination and seedling growth.

period. The lipid mobilization is regulated either by the factors influencing lipase activity directly or by the action of a thiole protease processing oleosin which enhances the organelle TAG matrix accessibility to lipolytic actions (Babazadeh et al., 2012). Lipase activities of seeds in several plant species, including rapeseed, were not active at the beginning of germination period and their activities rapidly increased afterwards (Rosales et al., 1998). The free fatty acid contents of cotyledons increased till 72 h, and then decreased. This may be due to the increase in the hydrolysis of oil, and then the conversion of free fatty acids to sucrose and their mobilization to the growing embriyonic axis. It has been reported that the biosynthesis of membrane lipids contributes significantly to the cell development and the rapid growth of growing embriyonic axis (Bewley and Black, 1983; Munshi et al., 2007; Satyanarayana et al., 2011).

The changes in fatty acid compositions (%) of cultivars during the study period were given in Tab. 2. The palmitic acid content slightly decreased at the end of the study period for both cultivars, however; the stearic acid contents of the cultivars increased during the same time period. The oleic acid contents of cultivars steadily decreased, while their linoleic and linolenic acid contents increased during the germination period. At 0 h, TR 3080 had an oleic acid content of 19.98%, and DUET CL had an oleic acid content of 89.95%. After 120 h, their oleic acid contents decreased to 18.33% and 85.19%, respectively. Linoleic acid contents increased from 67.06% to 69.12% for TR 3080 and from 1.77% to 5.75% for DUET CL. (Tab. 2). Cultivars have a high oleic and linoleic acid contents, therefore palmitic and stearic acid variations were not significant in the study, which was reported by other researchers for tobacco (Bush and Grunwald, 1972), mustard and crambe (Yaniv et al., 1998) and sunflower (Rabiei et al., 2007) seeds with low palmitic and stearic acids. Morever, Moya et al., (2000) reported that the changes in these fatty acids in mutant sunflower seeds with high palmitic and stearic acid contents were not significant for the study period. However, these fatty acids in cotton and soybean seeds were started to be utilized from the beginning of the germination and their contents decreased throughout the germination period (Joshi and Doctor, 1975; Joshi et al., 1973). During germination, the decrease of the oleic acid content and increase of the linoleic acid content may depend on the increased desaturase activity. It is known that oleic acid content decreased and linoleic acid content increased in seeds with high oleic or linoleic acid contents during germination (Bush and Grunwald, 1972; Joshi et al., 1973; Rosales et al., 1998; Moya et al., 2000; Rabiei et al., 2007). However, the changes in these fatty acids in mustard and crambe with low palmitic and stearic acid, and rapeseed with erusic acid were not significant (Yaniy et al., 1998). Linolenic acid content of cultivars slightly increased suggesting either the desaturation of fatty acids or the increase in relative amount of linolenic acid due to the breakdown of other fatty acids.

The dry matter content continually decreased during germination and early seedling development of sunflower cultivars, while the photosynthetic activity increased with the development of the cotyledons and leaves up to 96 h. Oil is utilized only at the later stages of seedling development. The breakdown of oil in the cotyledons coincided with an increase in total soluble and reducing sugar contents. The changes in the oil composition are marked by the increases of unsaturated linoleic and linolenic acids. A significant mobilization was also observed in total proteins which showed a decrease in the cotyledons associated with an increase in the free amino acid content. The results suggest that lipids, proteins, carbohydrates and color components contribute significantly to the seed germination and early seedling growth and development in studied sunflower cultivars.

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References

AOAC, 1990: Official methods of analysis. 15th Ed. Section. Association of Official Analytical Chemists, Washington D.C.

Babazadeh, N., Poursaadat, M., Sadeghipour, H.R., Hossein Zadeh Colagar, A., 2012: Oil body mobilization in sunflower seedlings is potentially regulated by thioredoxin H. Plant Physiol. Biochem. 57, 134-142.

Balasaraswatti, R., Sadasivam, S., 1997: Changes in oil, sugars and nitrogenous components during germination of sunflower seeds (*Helianthus annuus* L.). Plant Foods Hum. Nutr. 51, 71-77.

Bewley, J.D., Black, M., 1994: Seeds: physiology of development and germination. Plenum Press, New York, USA.

Bewley, J.D., Bradford, K.J., Hilhorst, H.W.M., Nonogaki, H., 2013: Seeds physiology of development, germination and dormancy. Springer Science+Business Media, LLC, 3rd Edition, ISBN 978-1-4614-4692-7.

Bush, P.B., Grunwalg, C., 1972: Sterol changes during germination of *Nicotiana tabacum* seeds. Plant Physiol. 50, 69-72.

Calucci, L., Capocchi, A., Galleschi, L., Ghiringhelli, S., Pinzino, C., Saviozzi, F., Zandomeneghi, M., 2004: Antioxidants, free radicals, storage proteins, puroindolines, and proteolytic activities in bread wheat (*Triticum aestivum*) seeds during accelerated aging. J. Agric. Food

Tab. 2: Changes in fatty acid compositions (%) of cultivars during germination and seedling growth.

Fatty acids	Cultivars	0 h	24 h	48 h	72 h	96 h	120 h
Palmitic acid	TR 3080	5.59	5.27	5.19	5.59	5.33	5.40
($C_{16:0}$)	DUET CL	3.25	3.24	3.22	3.24	3.11	3.12
Stearic acid	TR 3080	5.77	5.44	5.36	5.67	5.34	5.83
($C_{18:0}$)	DUET CL	2.78	2.85	2.73	2.76	2.80	2.86
Oleic acid	TR 3080	19.93	19.88	19.30	19.17	19.15	18.33
($C_{18:1}$)	DUET CL	89.95	89.83	89.50	88.48	86.36	85.19
Linoleic acid	TR 3080	67.06	67.77	67.83	68.11	68.13	69.12
($C_{18:2}$)	DUET CL	1.77	1.85	2.29	3.00	4.68	5.75
Linolenic acid	TR 3080	0.34	0.34	0.35	0.38	0.40	0.41
($C_{18:3}$)	DUET CL	0.22	0.22	0.23	0.26	0.59	0.66

Chem. 52, 4274-4281.

COLMENARES DE RUIZ, A.S., BRESSANI, R., 1990: Effect of germination on the chemical composition and nutritive value of amaranth grain. Cereal Chem. 67, 519-522.

DUBOIS, M., GILLES, K.A., HAMILTON, J.K., 1956: Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350-356.

ELMAKI, H.B., BABIKER, E.E., TINAY, A.H.E., 1999: Changes in chemical composition, grain malting, starch and tannin contents and protein digestibility during germination of sorghum cultivars. Food Chem. 64, 331-336.

GRAHAM, I.A., 2008: Storage oil mobilization in seeds. Annu. Rev. Plant Biol. 59, 115-142.

HARRIS, M., MACKENDER, O.R., SIMITH, D.L., 1986: Photosynthesis of cotyledons of soybean seedlings. New Phytol. 104, 319-329.

HARTREE, E.F., LOWRY, O.H., 1995: Detection and assay methods: Hartree-Lowry assay for quantitation of total protein. Wiley & Sons Inc., 1-24

JOSHI, A.C., CHOPRA, B.K., COLLINS, L.C., DOCTOR, V.M., 1973: Distribution of fatty acids during germination of soybean seeds. J. Am. Oil Chem. Soc. 50, 282-283.

JOSHI, A.C., DOCTOR, V.M., 1975: Distribution of fatty acids during germination of cottonseeds. Lipids 10, 191-193.

KIM, H.T., CHOI, U.K., RYU, H.S., LEE, S.J., KWON, O.S., 2011: Mobilization of storage proteins in soybean seed (*Glycine max* L.) during germination and seedling growth. Biochim. Biophys. Acta 1814, 1178-1187.

KIRK, J.T., ALLEN, R.L., 1965: Dependence of pigment synthesis on protein synthesis. Biochem. Biophys. Res. Co. 21, 523-530.

KOIWAI, A., MATSUZAKI, T., 1990: Changes in glycerolipid content and fatty acid composition during tobacco seed germination. Phytochem. 29, 73-76.

LARSON, L.A., BEEVERS, H., 1965: Amino acid metabolism in young pea seedlings. Plant Physiol. 40, 424-432.

LEE, P.Y., TAKAHASHI, T., 1966: An improved colorimetric determination of amino acids with the use of Ninhydrin. Anal. Biochem. 14, 71-77.

LOWRY, R.R., TINSLEY, I.J., 1976: Rapid colorimetric determination of free fatty acids. J. Am. Oil Chem. Soc. 53, 470-472.

MAQUARD, R., 1987: Qualitätsanalytik im Dienste der Ölpflanzenzüchtung. Fat Sci. Technol. 89, 95-99.

MILLER, J., RICE-EVANS, C., DAVIES, M.J., GOPINATHAN, V., MILNER, A., 1993: A novel method for measuring antioxidant capacity and its application for monitoring the antioxidant status in premature neonates. Clin. Sci. 84, 407-412.

MOYA, V.F., FORCE, E.M., GARCES, R., 2000: Metabolism of triacylglycerol species during seed germination in fatty acid sunflower (*Helianthus annuus* L.) mutants. J. Agric. Food Chem. 48, 770-774.

MUNSHI, S.K., SANDHU, S., SHARMA, S., 2007: Lipid composition in fast and slow germination sunflower (*Helianthus annuus* L.) seeds. Gen. Appl. Plant Physiol. 33, 235-246.

MUTO, S., BEEVERS, H., 1974: Lipase activities in castor bean endosperm during germination. Plant Physiol. 54, 23-28.

NASCIMENTO DO, R.S., SEIDL, P.R., HARRISI, R.K., 1994: Evidence for the formation of glucose (not sucrose) in the metabolism of germinating sunflower seeds. J. Agric. Food Chem. 42, 882-885.

NOCTOR, G., BERGOT, G.L., MAUVE, C., THOMINET, D., LELARGE-TROUVERIE, C., PRIOUL, J.L., 2007: A comparative study of amino acid measurement in leaf extracts by gas chromatography-time of flight-mass spectrometry and high performance liquid chromatography with fluorescence detection. Metabolomics 3, 161-174.

RABIEI, Z., TAHMASEBI ENFERADI, S., VANNOZZI, G.P., 2007: Regulation of polyunsaturated fatty acids accumulation and characterization of linolenic acid after germination of sunflower seed. Helia 30, 175-182.

ROSALES, M.P.R., KERKEB, L., FERROL, N., DONAIRE, J.P., 1998: Lipoxygenase activity and lipid composition of cotyledons and oil bodies of two sunflower hybrids. Plant Phsiol. Biochem. 36, 285-291.

SATYANARAYANA, B., SUBHASHINI DEVI, P., ARUNDHATI, A., 2011: Biochemical changes during seed germination of *Sterculi aurens* Roxb. Not. Sci. Biol. 3, 105-108.

SOMOGYI, M., 1952: Notes on sugar determination. J. Biol. Chem. 195, 19-23.

TARASEVIČIENĖ, Z., DANILČENKO, H., JARIENĖ, E., PAULAUSKIENĖ, A., GAJEWSKI, M., 2009: Changes in some chemical components during germination of broccoli seeds. Not. Bot. Horti Agrobot. 37, 173-176.

THEIMER, R.R., ROSNITSCHEK, I., 1978: Development and intracellular localization of lipase activity in rapeseed (*Brassica napus* L.) cotyledons. Planta 139, 249-256.

TONGUÇ, M., ELKOYUNU, R., ERBAŞ, S., KARAKURT, Y., 2012: Changes in seed reserve composition during germination and initial seedling development of safflower (*Carthamus tinctorius* L.). Turk. J. Biol. 36, 107-112.

VON LINTIG, J., WELSCH, R., BONK, M., GIULIANO, G., BATSCHAUER, A., KLEINIG, H., 1997: Light-dependent regulation of carotenoid biosynthesis occurs at the level of phytoene synthase expression and is mediated by phytochrome in *Sinapis alba* and *Arabidopsis thaliana* seedlings. Plant J. 12, 625-634.

WANASUNDARA, P.K.J.P.D., WANASUNDARA, U.N., SHAHIDI, F., 1999: Changes in flax (*Linum usitatissimum* L.) seed lipids during germination. J. Am. Oil Chem. Soc. 76, 41-48.

WEISS, E.A., 2000: Oilseed Crops, 2nd Edition, Blackwell Sci. Ltd., Victoria, Australia.

WILSON, K.A., 2006: Mobilization of storageproteins in dicots. In: Black, M., Bewley, J.D., Halmer, P. (eds.), Encyclopedia of seeds. Science, technology and uses, 672-674. CABI, Wallingford.

YANIV, Z., SHABELSKY, E., SCHAFFERMAN, D., GRANOT, I., KIPNIS, T., 1998: Oil and fatty acid changes in *Sinapis* and *Crambe* seeds during germination and early development. Ind. Crops Prod. 9, 1-8.

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