MOLECULAR ASPECTS AND REGULATION OF SUNFLOWER SEED

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ABSTRACT

Sunflower is a major oilseed crop, cultivated throughout the world, and the nutritional quality of its edible oil ranks among the best vegetable oils in agricultural product. In Tunisia, there is a lack of study on biochemical characterization of sunflower germplasm oil. Regular sunflower oil is rich in linoleic acid. The highest antiradical activity and FRAP were generally determined in older plants (mid-flowering and late flowering stages). To improve its properties for different applications several genotypes with modified fatty acid compositions have been developed. Amongst them, the most remarkable have been high oleic and high stearic types. High stearic sunflower lines reported to date have been produced by traditional methods of breeding and mutagenesis. The mutations affected the expression of enzymes responsible for stearate desaturation in developing seeds. This trait has been combined with standard and high oleic backgrounds, giving high stearic lines with high contents of linoleic or oleic acids and thus different physical properties, increasing their functionality and potential applications. Nevertheless, for applications requiring plastic or confectionery fats, the oils have to be oil fractionated to obtain derived fats and butters with higher levels of solids.

Keywords: *Oilseeds, sunflower oil, High stearic sunflower, bio-molecules, oil fractionation.*

INTRODUCTION

There is scientific evidence that the overproduction of reactive oxygen species (ROS) in cells of the body beyond those needed for the effectiveness of the antioxidant defense system may cause damage to such bio-molecules as lipids, proteins and DNA, and as a consequence may lead to various degenerative diseases, including cancer, diabetes mellitus, cardiovascular disease, hypertension, rheumatoid diseases, arthritis and neurodegenerative diseases. The consumption of antioxidants in food and dietary supplements has been linked to a reduced risk of these diseases. Antioxidants also play an important role in extending the shelf life of food. Utilized as additives, they limit the oxidation of food product ingredients, especially lipids. The increasing interest in new sources of natural antioxidants is thus justified, considering the above and general trend of using natural substances to replace synthetic ones. Sunflower is a short season plant that is native to North America and is currently grown worldwide. It is generally planted for seed and oil production purposes. Sunflower seeds are the fourth largest source of edible oil after soybean, rapeseed and peanut. In order to obtain good quality seeds, sunflowers should be harvested after reaching physiological maturity with a moisture content of about 10–13%. However, younger plants can also constitute valuable agricultural material. Green sunflower plants are used as forage and a silage source by livestock producers because of their nutritional quality, that is, high protein and fat contents.

LITERATURE REVIEW

Athika Rampadarath (2023) Type-2 diabetes mellitus (T2D) is one of the leading non-communicable diseases of global concern. Knowing the exact mechanism of action of available antidiabetic agents, particularly natural products, may assist in providing effective therapeutic solutions. The antidiabetic action of Helianthus annuus (sunflower) seed has been established; however, the molecular mechanism of action, especially the essential oil, is lacking. Thereby, we present an insight toward understanding the mechanism of the antidiabetic action of sunflower seeds via the stimulation of glucose to enhance insulin release.

Katarzyna Sulewska (2023) The profile of phenolic compounds changes during the growth of a plant and this change affects its antioxidant potential. The aim of this research has been to find the growth stage of flax with the highest antioxidant capacity, and to determine the phenolic compounds responsible for such a capacity. Coniferin, its derivative, and hydroxycinnamic acid derivatives were also detected. Most of the individual flavone C-glycoside contents in the extracts decreased when increasingly older plants were considered; however, the isoorientin content did not change significantly from the steam extension to the seed ripening stages.

Sana Medimagh (2022) Sunflower (Helianthus annuus) is a major oilseed crop, cultivated throughout the world, and the nutritional quality of its edible oil ranks among the best vegetable oils in agricultural product. In Tunisia, there is a lack of study on biochemical characterization of sunflower germplasm oil. Moreover, the classification of the evaluated sunflower accessions using clustering by Euclidean distance revealed four main groups. These data can be useful for selecting sunflower accessions and the development of varieties with improved oil quality.

Kandasamy Saravanakumar (2021) Traditional, complementary, and integrative medicine are globally accepted alternative methods for the treatment of diabetes mellitus (DM). However, the mechanism of anti-diabetic effects of Helianthus tuberosus L. remains unproven. In the present study, antioxidant and anti-diabetic activity of the tubers of H. tuberosus were studied in detail. Methanolic extracts of H. tuberosus tubers were subjected to solvent fractionation method by increasing the polarity of the solvent using n-hexane, and ethyl acetate. The obtained methanol extracts and its fractions were subjected to free radical scavenging activity (DPPH and ABTS assay) and in vitro enzyme (α-amylase and α-glucosidase) inhibition assay.

Michał A Janiak (2020) The aim of this study was to evaluate the differences in the antioxidant activity and phenolic profile of sunflower (Helianthus annuus L.) extracts obtained from the aerial parts of plants harvested at five growth stages. Phenolic compounds, such as mono- and dicaffeoylquinic acid isomers and caffeic acid hexose, were identified using the LC-TOF-MS/MS technique. The predominant compound during the growth cycle of the plant was 3,5-di-O-caffeoylquinic acid, whose content was the highest at the mid-flowering stage. The total phenolic content was also the highest in sunflowers at the mid-flowering stage.

Seed development and composition

Seed development in higher plants begins with a double fertilization process that occurs within the ovule and ends with a maturated seed primed to become the next

plant generation. The major events that occur during seed development. Embryo development can be divided up into two phases, the first or "embryogenesis" involves cell divisions associated with morphogenetic events which form the basic cellular pattern for the development of the shoot-root organs and the primary tissue layers, and it also programs the regions of meristematic tissue formation.

Following the cell division arrest at the end of the embryo growth phase, the seed enters the second phase, which is called "maturation phase", this process involves cell growth and the storage of reserves, such as proteins, starch and oils, required as 'food and energy supply' during germination and seedling growth.

Dormancy and control of germination

An internationally hierarchical system of classification for seed dormancy. The system has divided dormancy into five classes: (1) is physiological dormancy (PD), which contains three depth levels: deep, intermediate and nondeep. Moreover, depending on the PD depth level, dormancy can be released by different stratification treatments or GA treatment; (2) morphological dormancy (MD), which caused by a delay of embryo development; (3) morphophysiological dormancy (MPD), it is a combinational dormancy (PD + MD); (4) physical dormancy (PY) is due to the existing of water impermeable layers of palisade cells in the seed coat and it also can be released by mechanical or chemical scarification; and (5) combinational dormancy (PY + PD). However, inside all of these classifications, PD (non-deep level) is the most common kind of dormancy because it occurs in part of gymnosperms and in all major clades of angiosperms, and depending on the species, the dormancy alleviation correlated with stratification, scarification, a period of dry storage (after-ripening) or gibberellins (GAs) treatment. Recently, the works by Finch-Savage and Leubner-Metzger (2006) and Cadman et al. (2006) have provided insight into the molecular mechanisms of non-deep PD. Two terms of physiological seed dormancy have been distinguished; the intrinsic molecular mechanisms determined by seed components, namely embryo and coat dormancy.

Cellular events during germination

The dehydrated state of mature seed helps to withstand drought and extreme temperatures. Germination begins with water uptake by the dry seed during imbibition and ends with the embryonic axis or radicle elongation. During this process, a sequence of cellular events is initiated following seed water uptake which ultimately leads to emergence of the radicle and complete germination successfully. Metabolism commences in the seeds as soon as their cells are hydrated. Respiration and protein synthesis have been recorded within minutes of imbibition, using components conserved in the dry seed. This is followed by synthesis of RNA, and DNA repair and synthesis.

Temperature action on germination rates

The effect of temperature on dormancy breaking differs between dry and imbibed seeds. Perhaps the most extreme cases of dormancy breaking by temperature in dry seeds are seen in the actions of fire on the coats of hard, water-impermeable seeds. But the dry seeds more commonly may experience gradual dormancy through dry after-ripening, and moreover, this process which is enhanced as temperature increases, is most obvious in species with short life cycles whose seeds often exhibit

shallow dormancy, an attribute that allows rapid after ripening in warm summers enabling the production of more than one generation a season.

Sensitivity to temperature is correlated with depth of dormancy and is critical for winter and summer annuals to determine when they germinate. Seeds have an intrinsic upper limit temperature for germination, which is determined by environmental and genetic factors. In imbibed mature seeds, dormancy is frequently broken by a relatively low temperature (chilling) or in some species by warm temperatures.

METHODOLOGY

Sunflower and maize varieties were used to conduct preliminary germination tests with different concentrations of oxadiargyl. Healthy sunflower seeds and maize grains were surface sterilized using 2% sodium hypochloride solution for 2 minutes. In order to remove any excess chlorides present, it was washed repeatedly with distilled water 8-10 times. Sunflower seeds have a hard seed coat; hence to overcome dormancy the replicates were placed in the moist substratum and kept at a temperature of 10-20°C for germination for initial period of seven days. Ten comparably sized sunflower seeds and maize grains (sample of 100 seeds in each replicate) were separately placed in ten sterilized petri plates of 9cm containing different concentrations of herbicide solution. One set each was kept with distilled water which served as control. The test solutions were changed every alternate day till the termination day for both the crop varieties. Radical emergence from the seed and grain was taken as criteria for germination. Sunflower and maize require 7 days of incubation period for complete germination. During the study of preliminary screening of seeds, sunflower variety Morden and maize variety NAC-6004 showed maximum germination in control sets within the concentrations used when compared to other varieties. The two varieties of sunflower and maize were found to be resistant to the infection caused by fungus and were best suited for laboratory experiments with the herbicide. Hence these two varieties were taken into consideration for further experimental studies to be carried out.

RESULTS

Survey collection and identification of Diaporthe isolates

During the 3-year survey, two species of Diaporthe were isolated from infected sunflower stems sampled from the Northern Plains in the United States (Table 1). The frequency of D. helianthi and D. gulyae varied over the years among the three states. In 2010, D. helianthi was isolated at frequencies of 3.9% from samples collected in SD to 66.7% from samples collected in MN and 87.5% from samples collected in ND (Table 1). However, D. gulyae was isolated at frequency of 90.8% from samples collected in SD. In 2011, D. helianthi was isolated at frequencies of 73.0% from samples collected in MN and 34.8% ND to 84.3% in SD, while D. gulyae was isolated at frequency of 2.9% from samples collected in SD (Table 1). In 2012, only D. helianthi was isolated and at frequencies of 100% from samples collected in all the three states (Table 1).

Table 1. Isolates of Diaporthe spp. originating from the U.S. characterized into species using the ITS gene region

	Number of Diaporthe isolates

				recovered			
Year	State	Fields Surveyed	Total no. of stems collected	D. helianthi	D. helianthi isolation (%)	D. gulyae	D. gulyae isolation (%)
2010	MN	1	6	4	66.7	0	0.0
2010	ND	6	8	7	87.5	0	0.0
210	SD	48	76	3	3.9	69	90.8
2011	MN	14	37	27	73.0	0	0.0
2011	ND	14	23	8	34.8	0	0.0
2011	SD	23	70	59	84.3	2	2.9
2012	MN	10	26	26	100.0	0	0.0
2012	ND	5	14	14	100.0	0	0.0
20	SD	11	15	15	100.0	0	0.0
12							

Table 2. Isolates of Diaporthe spp. received from international collaborators (in 2011 and 2012) and characterized into species using the ITS gene region

Country	Year	Number of Fields	Number of Stalks	Number of Diaporthe isolates recovered			
				D.helianthi	D.helianthi isolation (%)	D. gulyae	D. gulyae isolation (%)
Russia	2011	7	7	5	71.4	0	0.0
Canada	2011	2	20	0	0.0	7	35.0
China	2011	3	7	0	0.0	0	0.0
Turkey	2012	3	7	0	0.0	0	0.0
Canada	20	2	2	0	0.0	0	0.0

	12						
Yugoslavia and Serbia	2012	9	9	2	22.2	0	0.0
Bulgaria	2012	1	1	1	100.0	0	0.0
Croatia	2012	12	12	12	100.0	0	0.0

From the international collections, the frequency of D. helianthi and D. gulyae varied over among four countries (Table 2). D. helianthi was isolated at frequencies of 22.2% from samples received from Yugoslavia and Serbia to 71.4% from Russia and 100.0% from Bulgaria and Croatia (Table 2). In contrast, D. gulyae was isolated only from samples received from Canada and at a frequency of 35.0% (Table 2).

Table 3. Diaporthe isolates and their mating type PCR reaction

Isolates[a]	Year of isolation	State	Species identity[b]	Detection of mating type genes[c]
				MAT 1-1-1
D6	2010	SD	D. gulyae	+
D9	2010	SD	D. gulyae	+
D12	2010	SD	D. gulyae	+
D14	2010	SD	D. gulyae	+
D21	2010	SD	D. gulyae	+
D32	2010	SD	D. gulyae	+
D40	2010	SD	D. gulyae	+
D25	2010	SD	D. gulyae	+
D45	2010	SD	D. gulyae	+
D48	2010	SD	D. gulyae	+
D86	2010	SD	D. gu	+

			lyae	
D20	2010	SD	D. gulyae	+
D35	2010	SD	D. gulyae	+
D47	2010	SD	D. gulyae	+
R_P131	2010	MN	D. helianthi	+
R_P129	2010	ND	D. helianthi	+
R_P132	2011	MN	D. helianthi	+
R_P126	2011	MN	D. helianthi	+
R_P121	2011	MN	D. helianthi	+
R_P105	2011	MN	D. helianthi	+
R_P140	2011	MN	D. helianthi	+
R_P137	2011	MN	D. heli	+
			anthi	
R_P134	2012	MN	D. helianthi	+
R_P118	2012	MN	D. helianthi	+
R_P139	2012	MN	D. helianthi	+
R_P107	2012	MN	D. helianthi	+

a. Isolates are a subset of 234 isolates, and were chosen as representatives for species-level identification.

b. Species identity was established based on phylogenetic analysis of the internal transcribed spacer region (ITS), elongation factor subunit 1-α (EF1α) and actin (ACT), conidial dimensions, and colony growth.

c. Mating type diagnosis was performed using the protocol of Santos et al. (2010).

Mating type identification of the U.S. Diaporthe isolates

The amplification reactions for MAT1-1-1 and MAT1-2-1 genes were performed, but the sequences were not used in phylogenetic analysis. The mating-type diagnosis using primers showed that all D. gulyae isolates in this study study have both MAT1-1-1 and MAT1-2-1 genes suggesting they are homothallic (Table 3). For D. helianthi isolates, only the MAT1-1 locus was detected (Table 3).

CONCLUSION

Since increasingly more consumers are becoming aware of agricultural practices

and their impact on the environment and food quality, pesticide toxicity on non-target crop species is a topic that needs to be investigated. Future line of work includes the following aspects. Experiments on field studies may be taken up to strengthen the observations of laboratory studies conducted under hydroponic conditions. Studies may be conducted on the permeability of the membrane and ion uptake. Herbicides are widely used to protect crops against weeds; nevertheless a massive introduction of these molecules into the fields can generate negative effects on the environment. They drastically influence all aspects of primary and secondary metabolism in crops when given to control undesired weeds. Further, a detailed investigation is required to strengthen the conclusion at cellular and molecular level and also on the persistence of herbicide at different soils. Based on the results obtained from the present investigation, oxadiargyl is responsible in affecting the seed germination which leads to inhibition of enzyme activity causing reduction in seedling growth.

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