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Research Note

Germination, vigour and dormancy of sunflower seeds following chemical desiccation of female plants

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Summary

The aim of this study was to analyse the impact of chemical desiccation on (i) the germination and vigour of seeds of contrasting sunflower genotypes, and (ii) the level of seed dormancy, with emphasis on the anatomical characteristics of seed coverings. Two treatments were applied, at 30% seed moisture content: (i) spraying plants with paraquat and (ii) detaching seed heads. Control untreated plants remained in the field until 10% seed moisture was reached. Seed dormancy (without pretreatment), germination (after applying one of three dormancy breaking treatments: pre-chilling, pericarp removal, pericarp + seed coat removal) and vigour (by electrical conductivity) were analysed. Chemical desiccation advanced harvest time by 35 - 43 days, increased seed dormancy and improved germination and vigour. Pericarp and seed coat removal had the greatest effect on overcoming dormancy. The outer cell wall thickness of the endospermatic cell layer of the seed coat was associated with dormancy level for the desiccation treatments.

Experimental and discussion

In seed production, chemical desiccation is widely used with the objective of advancing ripening. This technique reduces the losses caused by exposure to weather, pathogens and bird attacks, and improves harvest conditions. Desiccants vary in composition and mode of action, paraquat (salt of 1,1'-dimethyl-4,4'-bipyridinium) being the most commonly used chemical in grain crops. Even though sunflower seed producers are aware of the advantages of chemical desiccation, this topic has not been widely reflected in scientific literature. According to Liović *et al*. (2008), chemical desiccation could increase seed germination. However, Howatt *et al*. (2009) found reductions in seed germination depending on the seed moisture content. In addition, anticipated harvest has been reported to increase seed dormancy levels (Crnobarac, 1987). For this reason it is important to

assess the possible consequences of chemical desiccation on seed dormancy. Dormancy in sunflower is associated with hormonal processes, also accompanied by dormancy imposed by the pericarp and seed coat (Le Page-Degivry and Garello, 1992). The latter seems to be more important in wild biotypes than in commercial hybrids (Brunick, 2007). The sunflower seed (kernel) consists of embryo and a seed coat intimately coalesced with a single layer of endospermatic cells (Seiler, 1997). The mechanisms by which these structures impose dormancy are complex and not yet fully understood. Rolletschek *et al*. (2007) showed that oxygen diffusion to the embryo is limited by a lipid membrane which covers the embryo, but its location related to the other seed covering layers is not clear. Recently, Weiss *et al*. (2013) have shown that some characteristics of the seed coverings (apical suture of the pericarp and distance to radicle) could explain differences in gas and water exchange. These limitations could, in turn, affect the seed dormancy level. Thus, the anatomy of sunflower seed coverings could explain variations in levels of dormancy, especially if they are affected by chemical desiccation. The aim of this study was to analyse the impact of chemical desiccation on (i) the germination and vigour of contrasting sunflower genotypes, and (ii) the level of dormancy, with emphasis on the anatomical characteristics of seed coverings.

An experiment was carried out in Venado Tuerto, Argentina (33°44' S; 61°58' W) in 2011 on two sowing dates: 25 September 2011 (experiment 1) and 31 October 2011 (experiment 2). Two single-cross hybrids and one three-way hybrid with contrasting genetic backgrounds were used. Hybrids G1, G2 and G3 were produced by crossing the respective female line × restorer male (IL02 × IL06, IL01 × IL03 × IL07, IL04 × IL08, respectively). G1 and G2 are traditional low oleic acid hybrids and G3 is high in oleic acid. Plots were managed without nutritional limitations, pests, weeds or diseases. Rainfall was supplemented with drip irrigation to minimise water deficit.

Two treatments were applied: (i) chemical desiccation with paraquat (PAR) and (ii) detaching seed heads (CUT). The PAR treatment consisted of spraying the whole plant with 2 L ha^{-1} paraquat dissolved in water (1% v/v) using a knapsack sprayer equipped with hollow-cone nozzles with 3 bar pressure. Seed moisture was measured on a sample of 120 g seeds, randomly picked from three plants per plot, using a portable moisture meter. Paraquat was sprayed when developmental stage R7 was reached (according to Schneiter and Miller, 1981) 40 to 44 days after flowering (DAF) in experiment 1 when seeds had reached 27.4 ± 0.50% moisture content (wet basis, mean ± standard deviation) and 31 to 36 DAF in experiment 2 when seeds had reached 30.3 ± 0.57% moisture content. Such moisture contents are below sunflower physiological maturity (38% moisture content) and correspond to those commonly used by the global seed industry to anticipate harvest time. Plants were harvested four days after paraquat application, when brown leaves fell to the touch and seed moisture reached 25.8 ± 4.78% for experiment 1 and 25.0 ± 2.21% for experiment 2. The CUT treatment involved detaching seed heads from the mother plant at the same time as paraquat application for both experiments, which served to control possible chemical effects additional to desiccation. Heads were harvested at the same time as for the PAR treatments, when seed moisture was 23.7 ± 1.51% in experiment 1 and 28.4 ± 0.48% in experiment 2. Heads from PAR and CUT treatments were placed in an air-forced fluid bed dryer at room temperature for 48 hours, until seeds reached 7-10%

moisture content, which is safe for storage (Bewley and Black, 1994). Untreated control plants remained in the field until seeds reached 10 and 12% moisture content, averaging across genotypes, for experiments 1 and 2, respectively. Control plants remained in the field 35 and 43 days longer than the PAR and CUT treatments, for experiments 1 and 2, respectively. Seeds were removed using a mechanical hand thresher, cleaned using sieves, and stored in paper bags at room temperature (25°C) and 50-80% air relative humidity until analysed.

Four replicates of 50 seeds were used for each test one month after PAR, CUT and control harvest.

Viability: determined through a tetrazolium test (ISTA 2013).

Dormancy: seeds were placed in a germination chamber at 25°C without pre-treatment. As viability was over 99%, fresh seeds which failed to germinate but remained clean and firm on the 10th day after starting the test, were considered dormant.

Dormancy-breaking treatments: (i) pre-chilling for 48 hours at 5°C in water-saturated paper substrate (ISTA, 2013); (ii) manual pericarp removal (PR) after 18 hours of soaking using a scalpel; and (iii) pericarp and seed coat removal (PSCR), as for the PR treatment, but also removing seed coat tissues.

Germination: after dormancy-breaking treatments, seeds were incubated between paper substrate at 25°C with 12 hours light / 12 hours dark (ISTA, 2013). On the 10th day after starting the test, the seeds were classified as germinated (normal and abnormal seedlings), dead or fresh.

Vigour: determined through electrical conductivity (EC) test. PR seeds were placed into 75 ml distilled water at 25°C for 24 hours. Conductivity was measured according to Braz *et al*. (2008) and expressed as $\mu S\ cm^{-1}\ g^{-1}$.

Pericarp and seed coat tissues were separately fixed in FAA solution, embedded in paraffin, transversely sectioned at 10-15 µm with a Minot-type rotary microtome, and stained with safranin-fast green (Johansen, 1940). Digital fluorescence micrographs were made using a Zeiss Axioplan optical microscope. Digitised images were analysed using Image Tool 3.0 software (Wilcox *et al*., 2002) to quantify epidermis plus hypodermis thickness (E+H), schlerenchyma thickness (ST), number of schlerenchyma layers (N°SL), schlerenchyma maximum cell wall thickness (SMCWT), endospermatic cell thickness (CT), length (CL), outer cell wall thickness (OCWT) and inner cell wall thickness (ICWT). Experimental design was a split-plot with two replicates. ANOVA and LSD tests were performed. Percentage values were transformed using the angular transformation. Principal component analysis (PCA) was conducted on seed dormancy and anatomical data (biplot). Statistical software used for analysis was Infostat (www.infostat.com.ar).

Without dormancy-breaking treatments, seeds from PAR and CUT treatments in experiment 1 showed high dormancy (table 1), with genotypes G1 and G3 showing the highest levels (79-91%). A similar behaviour was observed in experiment 2, although with a greater data variability (84-100%; not shown). Plants from the PAR and CUT treatments reached conditions suitable for mechanical harvesting 35-45 days before the control, shortening the natural process of seed maturation. Since seed dormancy is gradually lost in the post-maturation period (time after physiological maturity) in sunflower (Bianco *et al*., 1994; Bazin *et al*., 2011) shortening of this period is expected to

Table 1. Seed dormancy, germination after pre-chilling, pericarp removal (PR) or pericarp and seed coat removal (PSCR), and vigour (electrical conductivity) of three sunflower hybrids under desiccation treatments (PAR = paraquat; CUT = detaching seed heads). Data are from experiment 1. Different lowercase letters indicate significant differences between desiccation treatments ($P < 0.05$) within each column. Values for ANOVA tests for genotype (G), desiccation treatments (T) and their interaction are also shown.

Hybrid	Treatment	Seed dormancy (%)	Germination (%) after dormancy-breaking treatments			Electrical conductivity ($\mu S\ cm^{-1}\ g^{-1}$)
			Pre-chilling	PR	PSCR	
G1	PAR	86 a	31 b	84 a	92 b	31 a
	CUT	91 a	15 c	78 a	100 a	27 a
	Control	13 b	77 a	85 a	90 b	30 a
G2	PAR	69 a	20 b	79 a	92 a	26 b
	CUT	59 a	33 b	74 ab	94 a	28 ab
	Control	6 b	75 a	57 b	84 b	39 a
G3	PAR	79 a	29 b	18 b	88 a	44 b
	CUT	84 a	12 b	28 b	78 b	44 b
	Control	8 b	66 a	72 a	69 b	52 a
G		0.0021	0.2911	< 0.0001	< 0.0001	< 0.0001
T		< 0.0001	< 0.0001	0.0370	0.0001	0.0289
G × T		0.1432	0.0801	0.0003	0.0040	0.2706

increase dormancy levels. Pre-chilling resulted in a lower proportion of normal seedlings while a high proportion of normal seedlings were observed after pericarp and seed coat removal (PSCR) (table 1). These different results in comparison with those of the PR method suggests a large influence of seed coat layers on dormancy levels, as has been suggested by Brunick (2007). The structure of the endospermatic cell layer was affected by PAR (figure 1). The outer cell wall thickness (OCWT), which increased 35% in PAR compared with the control, was associated with seed dormancy (figure 2). Therefore, the shortening of seed dehydration caused by CUT and PAR treatments resulted in an increased thickness of the cell wall. Effects of seed dehydration on cell wall structure have been observed in other grains (Rodriguez-Penagos and Black, 1994; Toole *et al*., 2010; Woodenberg *et al.*, 2014) but the present work is the first report for sunflower. It is still uncertain if the effect on sunflower seed dormancy is specifically due to the cell wall thicknesses, to their lipid nature (Rolletschek *et al*., 2007) or to both. A detailed study would be required to understand in how the rest of the seed coverings (live and non-live tissues) and their chemical composition affect sunflower seed dormancy.

Based on the results from the PSCR method for breaking dormancy, PAR and CUT treatments increased seed germination showing different sensitivity between genotypes, with G1 and G3 being the most sensitive, and G2 the least (table 1). Seed conductivity values ranged 26-52 $\mu S\ cm^{-1}\ g^{-1}$ among treatments and genotypes (table 1). Significant effects of genotype and chemical desiccation were observed, without significant interaction. Control treatment for G2 and G3 genotypes showed significantly higher

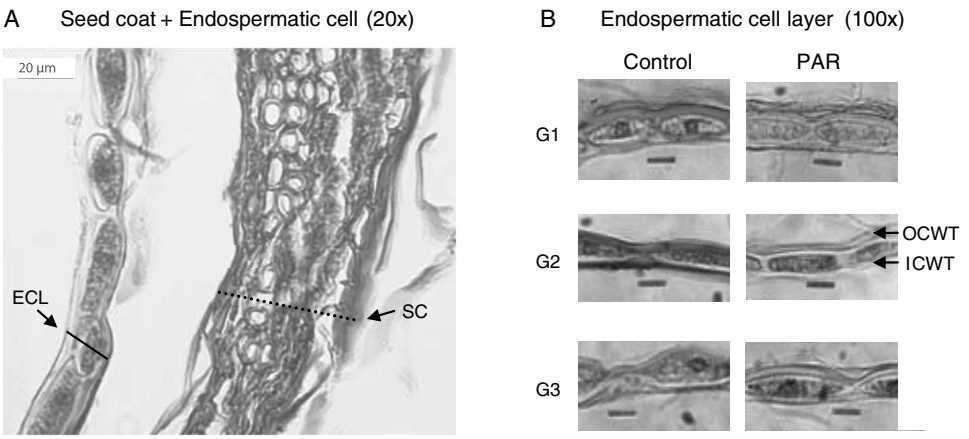

Figure 1. Anatomical characteristics of seed coat from sunflower hybrids (G1 to G3) under chemical desiccation treatments for experiment 1. (A) Seed coat from mature seed of control treatment (G1 hybrid) including inner endospermatic cell layer of live cells facing the embryo, and outer cell layers (mainly sclerotic or crushed) facing the pericarp. Bar = 20 µm. (B) Detail of the endospermatic cell layer under chemical desiccation (paraquat, PAR) and untreated control, with different thickness of outer and inner cell walls. Bar = 7 µm. Abbreviations: ECL, endospermatic cells layer; SC, seed coat; OCWT, outer cell wall thickness; ICWT, inner cell wall thickness.

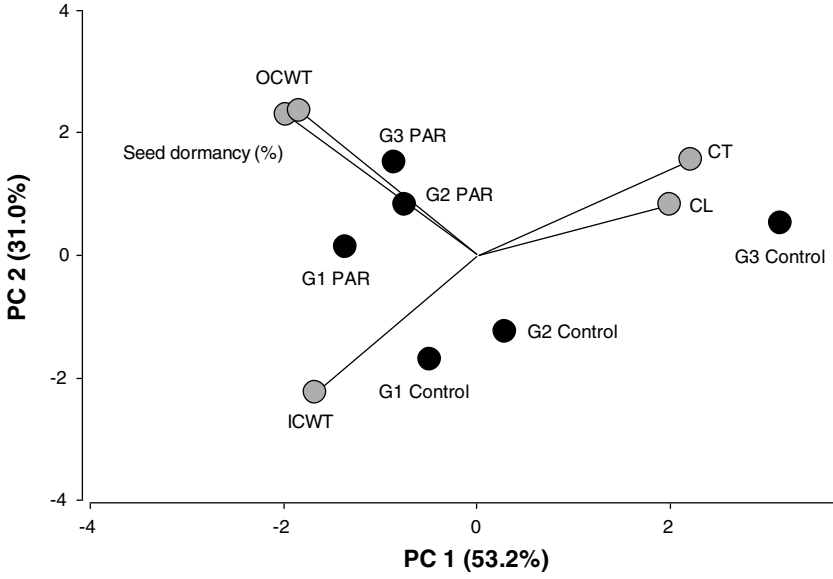

Figure 2. PCA (biplot) for seed dormancy and anatomical characteristics of seedlings from sunflower hybrids (G1 to G3) under chemical desiccation treatments for experiment 1. Abbreviations: OCWT, outer cell wall thickness; ICWT, inner cell wall thickness; CT, cell thickness; CL, cell length; PAR, paraquat.

conductivity values (suggesting lower vigour) with respect to PAR, while for G1 these differences were not significant. G3 exhibited the highest EC, indicating that the high oleic hybrid G3 had low seed vigour.

Positive effects of chemical desiccation on seed germination were observed by Liović *et al*. (2008) and Howatt *et al*. (2009) when desiccants were applied at low seed moisture content (< 50%). The increase in germination and vigour due to chemical desiccation may be related to the fact that this treatment limited the exposure to extreme climatic conditions, especially days with maximum temperature above 30°C (up to 20 days; data not shown) and this could have minimised seed deterioration prior to harvest. Differences in the genetic constitution of the hybrids could underlie the sensitivity to chemical desiccation between genotypes, since G2 is a three-way cross-type and the hybrid vigour from a single-cross female parent could be expressed in seed germination performance. Kannababu and Karivaratharaju (2000) reported high maternal contribution to the physiological quality of sunflower seeds. Further studies should explore a broad range of hybrids including simple and three-way cross-types of modern cultivated sunflower, tested in a range of environmental conditions, to evaluate maternal effects on physiological seed quality.

For the harvest conditions used in this study, chemical desiccation advanced harvest time by 35 - 43 days and increased seed dormancy. After seed coat removal a high proportion of normal seedlings were observed, indicating a large influence of this tissue on dormancy levels in sunflower. For desiccation treatments, the outer cell wall thickness of the endospermatic cell layer of the seed coat was associated with dormancy level. Chemical desiccation improves sunflower seed germination and vigour, with different sensitivity between genotypes.

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