Effect of abscisic acid on desiccation tolerance in rapeseed (*Brassica napus* L.) cultivars using somatic embryogenesis

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Introduction

Aspects of zygotic embryo maturation such as reserve products accumulation and acquisition of desiccation tolerance are shown to be influenced by ABA (Schmidt *et al.* 2005). In zygotic embryos, it was reported that ABA levels decreased during embryo development whereas ABA sensitivity increased during the same period. However, both endogenous ABA levels and sensitivity decreased during desiccation (Finkelstein *et al.* 1985). Studies showed that ABA level in somatic embryos are much lower than their zygotic counterparts (George *et al.* 2008). ABA treatments are shown to increase normal somatic embryo production (Feher *et al.* 2003). ABA treatments also shown to stimulate the production of protein and oil reserves in somatic embryos to levels comparable to those found in their zygotic counterparts (Schmidt *et al.* 2005). Desiccation is the final phase of normal embryonic development in most angiosperms and appears to be important in the transition from embryogeny to the ability to germinate and form normal seedlings (Bomal *et al.* 2002). The main difference of somatic and zygotic embryos is their difference in responding to desiccation. Zygotic embryos of orthodox seeds are desiccation tolerant whereas somatic embryos, either orthodox or recalcitrant, are desiccation sensitive.

Desiccation studies in rapeseed (*Brassica napus L.*) are of great importance. It had been shown that, in oilseeds such as rapeseed, at least 10% of the major storage product of developing embryos, which is triacylglycerol is lost during the desiccation phase of seed development (Chia *et al.* 2005). So a better understanding of this critical phase in seed maturation is needed if increasing in overall oil production of rapeseed is favored. Apart from this, it is possible that desiccated somatic embryos can be preserved successfully for long periods of time without using cryoprotectants pretreatments and expensive programmable freezers. So this simple method may provide a new tool for preservation of somatic embryos in gene banks.

In the present study, the effects of different ABA treatments and different drying rates on acquisition of desiccation tolerance in *Brassica napus* (cultivar *Opera*) were studied. Here, we tried to propose the proper dose of ABA and drying rate for inducing desiccation tolerance in these somatic embryos.

Material and Methods

Mature somatic embryos of cultivar *Opera*, which were formed in proper ABA treatments were chosen for desiccation treatments. Desiccation treatment 1 (D1) was a treatment without desiccation. In D1, embryos were directly transferred to Petri dishes containing 1/2MS basal media without PGRs. These Petri dishes were incubated at 22 ^{0}C in the light (3000 lux) generated by fluorescent lamps. Desiccation treatment 2 (D2) was fast desiccation treatment. In D2, embryos were dried within 4 hours by placing Petri dishes containing these embryos without covers in an air-flow cabinet. The relative humidity (RH) of the air flow was approximately 40%, as monitored with a hygroscope. In desiccation treatment 3 (D3) embryos were dried through a series of desiccators in which relative humidity (RH) was kept constant using a saturated solution of K_2SO_4 (97%), NaCl (75%) and $K_2CO_3.1.5H_2O$ (40%). Desiccators were kept in dark at 22 ^{0}C. In this desiccation treatment, embryos were transferred from a desiccator at a higher RH to one at a lower RH, every 72 hours. In order to avoid imbibitional damage, before rehydration, desiccated embryos from D2 and D3 were transferred to a desiccator in which the RH was kept 97% for 4 hours. After that, these embryos were cultured in 1/2MS basal media without PGRs (Sun *et al.* 1998), in the light (3000 lux) generated by fluorescent lamps at 22 ^{0}C. These embryos were assessed for desiccation recovery and normal development at 4 weeks after rehydration. Only those embryos that showed both shoot and root meristem growth were scored as surviving.

Table 1. Dose and application time of abscisic acid.

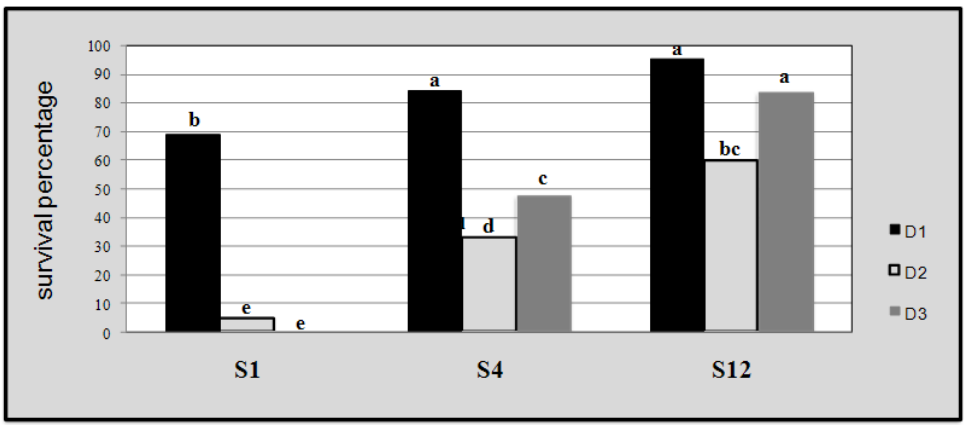
21-30	11-20	0-10	ABA treatment / Days
0	0	0	S1
0.5µM	0.5µM	0.5µM	S2
0	0	0.5µM	S3
0	0.5µM	0	S4
0.5µM	0	0	S5
10µM	10µM	10µM	S6
0	0	10µM	S7
0	10µM	0	S8
10µM	0	0	S9
50µM	50µM	50µM	S10
0	0	50µM	S11
0	50µM	0	S12
50µM	0	0	S13

The experiments were arranged in two factorial experiments as completely randomized designs. In each experiment, each treatment combination was replicated three times. Statistical analyses were done using SAS (version 9.00) analysis of variance procedures. Means of main effects interactions were compared by Tukey's Honestly Significant Difference Test of MSTATC software.

Results and Discussion

Both ABA and desiccation treatments had significant effects on induction of desiccation tolerance in *Opera*. In this experiment it had been shown that in the presence of exogenous ABA, germination percentage of somatic embryos increased significantly, no matter they were dehydrated or not. For example, comparing to the control, when ABA was used (either 0.5 µM or 50 µM), germination percentage increased nearly 15-25% in those embryos which were not dehydrated (Fig. 1). When mature somatic embryos were not dehydrated, no significant difference in germination percentage was observed between different ABA concentrations. In contrast, when they were dehydrated (either by fast drying or slow drying), significant difference were observed between ABA concentrations (Fig. 1).

Figure 1. Somatic embryo survival percentage after ABA treatments, desiccation treatments and rehydration in rapeseed (*Brassica napus cv. Opera*). Values represent the mean of three replicates. Means followed by the same letter are not significantly different (Tukey's Honestly Significant Difference Test, Probability level= 0.05).



Comparing to the control, germination percentage of fast dried somatic embryos increased 28% when 0.5 µM ABA was used, but it increased even higher (nearly 55%) when 50 µM ABA was used.

In the case of slow drying rate, germination percentage of these somatic embryos increased 48 and 84%, respectively, when 0.5 µM and 50 µM ABA were used. As shown in these cases, higher concentration of ABA was more beneficial on inducing desiccation tolerance than lower ABA concentration. Studies exhibited that the external surface of the desiccation-tolerant embryos was uniformly shriveled due to severe desiccation and their internal tissue system was well preserved. However, in desiccation-sensitive ones, dehydration caused tearing of the epidermis and collapse of the internal tissue system. After rehydration, desiccation tolerant embryos recovered and continue normal growing but desiccation sensitive ones did not recover or remained shriveled. When 0.5 µM ABA was used, in slow dried somatic embryos germination percentage was increased nearly 15 percent comparing to fast dried ones (Fig. 1). Furthermore, in slow dried somatic embryos which were formed in medium containing 50 µM ABA, germination percentage increased even higher (24%). In the absence of ABA, slow drying protocol led to complete intolerance to desiccation; whereas some survival occurred in the faster drying rate (Fig. 1). It is likely that another effect of ABA appears in such cases; if slow drying be considered as a form of accelerated ageing treatment; ABA might have reduced cellular damage by curtailing metabolic activity. As a result, somatic embryos could be more sensitive to slow drying when they are grown in the absence of ABA.

The results of this experiment showed that ABA treated embryos were more desiccation tolerant than non ABA treated embryos. Moreover, as the concentration of ABA increased, embryo tolerance to desiccation increased. Slow drying rate was more beneficial for desiccation tolerance induction than fast drying in ABA treated embryos. In addition, ABA treated embryos had higher germination rates even when they were not dehydrated.

Acknowledgment

The authors are gratefull to the University of Tehran for providing financial support of this study.

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