การเพิ่มความมีชีวิตของเมล็ดสังเคราะห์พริกหวาน โดยใช้กรดแอบซิสสิค

Enhancing the Viability of Sweet Pepper Synthetic Seed Using Abscisic Acid

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Abstract: The somatic embryos obtained from callus culture were treated with 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 and 1 mg/l ABA in MS medium (maturation formula) for 21 days. Then the seed encapsulation and the synthetic seed dehydration were pursued until the seeds lost 80 percent of their moisture contents. The results showed that the survival percentages were 33, 36, 47, 43, 55, 73, 83 and 37, respectively. Therefore, somatic embryos treated with 0.5 mg/l ABA improved survival rate of synthetic seeds dramatically. The ABA treated somatic embryos encapsulated with 3 percent w/v sodium alginate and 75 mM calcium chloride could prolong their germination for 6 weeks after storage at 25±2°C, with 16 hours photoperiod. The 6 weeks stored synthetic seeds had 63% survival rate after 6 days planting in MS media. The experimental results suggested the possibllity of ABA for germination and storage improvement of dry sweet pepper synthetic seeds.

Keywords: Sweet pepper, synthetic seed, abscisic acid, seed encapsulation

บทคัดย่อ: เมื่อเพาะเลี้ยงแคลลัสพริกหวานจนได้โซมาติกเอมบริโอแล้ว นำมาทดสอบด้วย ABA ความเข้มข้น 0 0.05 0.1 0.2 0.3 0.4 0.5 และ 1 มก./ล. ในอาหาร MS สูตรพัฒนาเอมบริโอให้แก่เป็นเวลา 21 วัน ผลิตเป็นเมล็ดสังเคราะห์ และทำการระเหยน้ำออกจากเมล็ดสังเคราะห์จนสูญเสียความชื้น 80 เปอร์เซ็นต์ ผลที่ได้คือ เมล็ดสังเคราะห์มีเปอร์เซ็นต์ความมีชีวิต 33 36 47 43 55 73 83 และ 37 เปอร์เซ็นต์ ตามลำดับ ดังนั้นการใช้ ABA 0.5 มก./ล. ในการเพาะเลี้ยงโซมาติกเอมบริโอ จะช่วยเพิ่มเปอร์เซ็นต์ความมีชีวิตของเมล็ดสังเคราะห์ได้มากขึ้นอย่างเห็นได้ชัด หลังจากนั้นนำโซมาติกเอมบริโอที่ผ่านการชักนำด้วย ABA ดังกล่าว มาเคลือบด้วย sodium alginate 3 %w/v และ calcium chloride 75 mM แล้วเก็บรักษาไว้ที่อุณหภูมิ 25±2 °C สภาพแสง 16 ชั่วโมง พบว่าสามารถเก็บรักษาเมล็ดสังเคราะห์ได้ยาวนานถึง 6

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สัปดาห์ และเมื่อนำเมล็ดสังเคราะห์ที่เก็บรักษาไว้ 6 สัปดาห์ มาปลูกในอาหาร MS จะใช้ระยะเวลาในการงอก 6 วัน เมล็ดสังเคราะห์พริกหวานที่ได้จากการทดลองนี้ ยังคงมีเปอร์เซ็นต์ความมีชีวิตสูงถึง 63 เปอร์เซ็นต์ จากผลการทดลองได้ชี้ให้เห็นถึงความเป็นไปได้ในการช่วยเพิ่มความมีชีวิต และความสามารถในการเก็บรักษาให้สูงขึ้นในเมล็ดสังเคราะห์พริกหวานแบบแห้งโดยใช้ ABA

คำสำคัญ: พริกหวาน เมล็ดสังเคราะห์ กรดแอบซิสสิค การเคลือบเมล็ด

Introductin

F1 hybrid of sweet peppers are not only expensive, but they are also produced in low number from many crosses due to problems of inter specific incompatibility and F1 hybrid sterility. (Harini and Sita, 1993). Moist synthetic seeds had many weak points. One of those was; they should be stored in the condition of low temperature, which was problematic in storage. Moreover, the seeds had short storage period while their conversion rate was also low. There was high respiration rate of somatic embryos in hydrogel, causing moist synthetic seeds to dry quickly in room temperature (Redenbaugh *et al*., 1987). It was found that dry synthetic *Brassica* spp. seed production with ABA to induce desiccation tolerance before dehydration could enhance the longer synthetic seeds storage up to 6 months without controlling temperature and moisture during the storage. It could also be germinated in soil and it had seedling comparing to moist somatic embryos (Takahata *et al*., 1993). ABA took role in somatic embryo development by defending precocious germination, inducing desiccation tolerance and deteriorating chlorophyll in order to decrease oxygen production (Elstner, 1982). Thus, the objectives of this research were; a) to use abscisic acid (ABA) induced desiccation tolerance of sweet pepper, b) to improve sweet pepper synthetic seed production technique for higher rate of germination and viability and c) to improve technique for prolonging sweet pepper synthetic seeds storage.

Materials and Methods

The protocol for indirect somatic embryogenesis of sweet pepper from mature zygotic embryos had been already established (Buyukalaca and Mavituna, 1996). This study, therefore, proceeded further experiments using somatic embryos as followed:

Experiment 1: Analyzing appropriate growth stage of somatic embryos in induce desiccation tolerance by using ABA.

Somatic embryos at late torpedo stage were transferred to 50 ml MS liquid medium which was the maturation formula containing 0.5 mg/l ABA for 3, 6, 9, 12, 15, 18, 21, 24 and 25 days in the dark, placing on a rotary shaker at 100 rpm at 25±2 °C (Buyukalaca and Mavituna, 1996). Synthetic seeds were then produced from somatic embryos before dehydration was pursued until the seeds lost 80% of their moisture contents. The next process was to test the survival rate and speed of germination by culturing at 25±2 °C and 16 hours/day photoperiod for one week. The survival percentage and speed of germination were recorded. This process was repeated three times, ten synthetic seeds for each.

Experiment 2: Analyzing appropriate ABA concentration to induce desiccation tolerance before synthetic seeds production.

Somatic embryos at late torpedo stage were transferred to 50 ml MS liquid medium which was the maturation formula containing ABA 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5 and 1.0 mg/l concentration, respectively for 21 days in the dark, placing on a rotary shaker at 100 rpm at 25±2 °C. Synthetic seeds were then produced from somatic embryos before dehydration was pursued until the seeds lost 80% of their moisture contents. The next process was to test the survival rate and speed of germination by culturing at 25±2 °C and 16 hours/day photoperiod for one week. The survival percentage and speed of germination were recorded. This process was repeated three times, ten synthetic seeds for each.

Experiment 3: Testing synthetic seeds germination after storage.

Somatic embryos from the maturation medium containing 0.5 mg/l ABA_for 21 days in the dark, placing on a rotary shaker at 100 rpm at 25±2 °C. Synthetic seeds were then produced from somatic embryos before dehydration was pursued until the seeds lost 80% of their moisture contents. They were stored in 250 ml Erlenmayer flask, 5 seeds for each then sealed with parafilm storage at 25±2 °C and 16 hours/day photoperiod for 0, 1, 2, 3, 4, 6 and 8 weeks, respectively. After that, those seeds, which were stored in different length of storage, were rehydrated and the final process was to test the survival rate and speed of germination by culturing at 25±2 °C and 16 hours/day photoperiod for one week. The survival percentage and speed of germination were recorded. This process was repeated three times, ten synthetic seeds for each.

Statistical analysis

All experiments were arranged in a completely randomized design with 3 replicates per treatment (10 embryos/replicate). Data was analyzed using analysis of variance and mean comparisons made by protected least significant difference at the 5% level of probability.

Results and Discussion

The results from experiment 1 was; Culturing 21-day-old embryos was found to 93 % survival of all seeds while there were only 4 % of abnormal seedling. The germination took only 3 days and its germination percentage decreased in more than 21-day-old embryos, which could be because the longer the accumulation of ABA, the more stimulation of somatic embryos into dormancy stage (Table 1).

The results from experiment 2 suggested that increasing ABA concentration gradually could increase survival percentage. On the other hand, seeds were variable which might due to the effect of ABA to emzyme activity in some plants. 0.5 mg/l ABA would lead to as much as 83% synthetic seeds survival while there were only 4 % of abnormal seedling, comparing to survival without ABA which synthetic seeds germinated only 33% and had as much as 13 % abnormal seedling. However, survival percentage would decrease again when the concentration of ABA was more than 0.5 mg/l because the very high ABA concentration resulted more in dormancy than inducing desiccation tolerance (Table 2)

The results from experiment 3 suggested that survival percentage would decrease every week and so as the speed of germination. However, the

Table 1 Result of growth period on synthetic seed survival percentage and speed of germination after dehydration was pursued until seeds lost 80% of their moisture contents.

Growth period (day)	% Survival (Normal seedling)	Abnormal seedling (%)	Speed of germination (day)
3	$0^{h\ 1/}$	0^{g}	-
6	0^{h}	0^{g}	-
9	17^{fg}	3^{fg}	7^{d}
12	14^{g}	16^{bc}	6^{c}
15	46^{e}	17^{ab}	6^{c}
18	76^{bc}	4^{ef}	4^{b}
21	93^{a}	4^{ef}	3^{a}
24	73^{c}	10^{d}	4^{b}
25	56^{d}	14^{cd}	6^{c}
CV (%)	16.73	13.18	8.84

[1/]Numbers with the English alphabets in the same column represents the statistic significantly different (P<0.05).

Table 2 Effect of ABA concentration on synthetic seed survival percentage and speed of germination after dehydration was pursued until seeds lost 80% of their moisture contents.

ABA (mg/l)	% Survival (Normal seedling)	Abnormal seedling(%)	Speed of germination (day)
0	$33^{h\ 1/}$	13^{bc}	3^{a}
0.05	36^{gh}	10^{cd}	3^{a}
0.1	47^{de}	6^{ef}	4^{b}
0.2	43^{e}	10^{cd}	4^{b}
0.3	57^{c}	9^{de}	4^{b}
0.4	73^{b}	4^{f}	4^{b}
0.5	83^{a}	4^{f}	4^{b}
1.0	37^{fg}	16^{ab}	5^{c}
CV (%)	13.61	14.22	7.29

[1/]Numbers with the English alphabets in the same column represents the statistic significantly different (P<0.05).

survival percentage would decrease obviously in the eighth day of storage. The survival percentage decreased to 43% while there were 10% abnormal seedling at that time. Considering the result in Table 3, the most suitable synthetic seed storage period was 6 weeks with 63 % survival and 7 % abnormal seedling. This was considered to be the satisfied percentage. Nevertheless, Binzel *et al*. (1996) suggested that the viability would increase more if applying 3.8 μM ABA before encapsulation with

Table 3 Result of storage time on inducing desiccation tolerance synthetic seed germination percentage and speed of germination after dehydration was pursued until seeds lost 80% of their moisture contents.

Storage time (week)	% Survival (Normal seedling)	Abnormal seedling(%)	Speed of germination (day)
0	93$^{a\ 1/}$	3^{d}	3^{a}
1	83^{b}	7^{cd}	3^{a}
2	83^{b}	3^{d}	3^{a}
3	67^{cd}	13^{ab}	4^{b}
4	66de	7^{cd}	5^{c}
6	63^{e}	7^{cd}	6^{d}
8	43^{f}	10^{bc}	6^{d}
CV (%)	10.13	13.82	6.52

[1/]Numbers with the English alphabets in the same column represents the statistic significantly different (P<0.05).

sodium alginate and dehydration. This leaded to 57 % synthetic seed survival. The storage by inducing desiccation tolerance with 0.5 mg/l ABA was done for 1, 2 and 3 weeks whose results were 48, 47 and 32 % of germination, respectively. Therefore, this result demonstrates the possibility of inducing desiccation tolerance by applying ABA into somatic embryos, conforming with Takahata *et al*. (1993) who could produce the most inducing desiccation tolerance when applying 100 μM ABA, developing into 27-48 % plantlets.

Conclusion

Use of ABA 0.5 mg/l in dried synthetic seeds production did improved their survival rate dramatically, which was higher in percentage than previous researches. This technique was also able to prolong synthetic seed at 25±2°C, resulting in low expense for the storage. Moreover, this technique can further be applied for other plants in order to increase yields in limited crop fields.

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