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Catatan Penelitian (Research Note)
Production of Seedling of *Carica papaya* L. by *Carica parviplora* (A.DC) Solms. Interspecific Hybrids Using Embryo Rescue

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ABSTRACT

A method of embryo rescue to produce seedlings of Carica papaya x Carica parviflora (A. DC) Solms. interspecific hybrids has been developed. Liquid medium of 0.5 Murashige and Skoog nutrients containing kinetin (0.25 μM) and NAA (0.25 μM) was the best medium to germinate the hybrids and produced the biggest leaf area index of the hybrids. Liquid medium of 0.5 MS nutrients containing kinetin (1.0 μM) and NAA (0.25 μM) produced the highest number of leaves of the hybrids, and produced the longest length of hypocotyl of the hybrids.

Key words : Carica papaya L., Carica parviplora (A.DC) Solms, Interspecific hybrids, Embryo rescue

INTRODUCTION

Carica parviflora (A. DC.) Solms is a wild relative of papaya which has pink flowers and small green fruit with pink ridges which, unlike papaya are inedible (Badillo, 1971). Interspecific hybrids of papaya x *C. parviflora* (A. DC.) Solms. may have novel ornamental value since they are likely to produce attractive pink-red flowers (a trait from *C. parviflora* (A. DC.) Solms.) and bigger and perhaps edible fruit (a trait from *C. papaya* L.) (Drew, personal communication).

Mekako and Nakasone (1975) reported that the success of wide hybridization using *Carica* spp is dependent, in part on the species used in the cross and the need for the plants to be in excellent health prior to the hybridization attempt. When a warm growth temperature and a low soil moisture conditions led to cross failure. Furthermore, Manshardt and Wenslaff (1989) found that the genotype of the female parent used for the hybridization attempt had a significant effect on the degree of success. Only those cultivars that could adequately nourish the developing embrio could be succesfully used as the female parent. By taking these concepts into mind successful crosses have been obtained between papaya and *C. monoica, C. parviflora, C.* pubescens, *C. quercifolia* and *C. stipulata*, while in only one case (papaya by *C. parviflora*) did the female genotype not matter. Eventhough papaya x *C. parviflora* (A DC.) Solms hybrids have been produced, very little viable seed was produced and this could not be converted into plants. Attempts to graft the hybrids shoots onto papaya root stock mature hybrid plants of papaya x *C. parviflora* has not been previously achieved.

To obtain the highest possible number of hybrid plants, it is important to understand the flora biology of both parents to be involved in the cross. For example, female flowers of *C. parviflora* (A. DC.) Solms. take 27 days to develop from bud emergence to anthesis while female flowers of papaya take 45 to 47 days to undertake the same changes. In addition, flowers of *C. parviflora* (A. DC.) Solms. undergo daily anthesis shortly after 6 am, reaching a peak between 8 and 10 am while papaya on the other hand undergo anthesis between 8 and 9 am. If these features of the floral biology are not taken into account then it is obvious that the crosses will fail (Mekako and Nakasone, 1975).

Earlier studies have shown that if hybrid zygotic embryos developed from crosses involving papaya and *C. parviflora* (A. DC) Solms. were not isolated before they had become 120 days old they would die in the fruit. Even when embryo rescue is used to save these hybrid embryos there is still a problem getting these immature structures to germinate (Mekako and Nakasone, 1975). An embryo rescue system has been developed for papaya x *C. cauliflora* Jacq. hybrids (Manshardt and Wenslaff, 1989). This system used basal MS nutrients containing BA (0.8 μM), NAA (2.6 μM) and agar (0,8%). More recently, Magdalita (1997) developed an improved technique using 0.5 De Forssard

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nutrient medium supplemented with GA_3 (10 µM), BAP (0.25 µM), NAA (0.25 µM), sucrose (58 mM) and agar (8 gL^{-1}). For seedling growth, another medium has been developed, which has similar make up but with a higher concentration of kinetin (1 µM). This higher ratio of cytokinins to auxins in this medium improved the hybrid seedling is leaf area and promoted their development (Magdalita *et al.* 1997).

In this paper, methods were developed for the production of papaya x *C. parviflora* (A DC.) Solms. hybrid seedlings by wide hybridization and embryo rescue. Thus the specific aims were to a) utilize an already existing method for the wide hybridization of papaya x *C. parviflora* (A DC.) Solms. to mass produce hybrid zygotic embyos, b) to utilize an already existing method for the embryo rescue and germination of these hybrid zygotic embryos *in vitro*

MATERIAL AND METHODS

Explant Material

Female and male *Carica papaya* plants, derived from nodal culture and *C. parviflora* (A DC.) Solms. female, and male seed-derived plants were used as parents in these summer 1994-1996 in Redlands Research Centre of Queensland Department of Primary Industries, Australia.

Wide Hybridization Techniques.

The first wide hybridization attempt was in the spring (October) of 1994 and continued into the summer (February) of 1995. The second wide hybridization attempt was made in the early summer (November) of 1995 and continued into the summer (February) of 1996.

Pollination of papaya plants was undertaken with pollen from *C. parviflora* and vice versa. This procedure was undertaken only when the male plant had reached the mature bud stage of flower development, just prior to anthesis. When *Carica papaya* was used as the female parent, only one terminal pistillate bud was pollinated, with the two subtending ones removed. Male flowers of *C. parviflora* (A DC.) Solms. were harvested in the morning (8.00 am.) and covered with white paper bags. Pollination was carried out at 8.30 am on the same day. To do this pollen of *C. parviflora* (A DC.) Solms. was brushed directly from the male flower onto the pistil of *C. papaya* flowers. The pollinated bud was covered with a white paper bag and closed around the petiole with a metal rim tag. When *C. parviflora* was used as female parent, the *C. papaya* male flower buds were collected at 7.00 am and pollen brushed directly onto the female flower of *C. parviflora*. The flowers were then enclosed in white paper bags and closed with a paper clip at the base.

The Embryo Rescue Technique.

Using technique of Magdalita (1997) rescue of the viable hybrid embryos produced in the crossed involving C. papaya x *C. parviflora* (A. DC.) Solms was undertaken in the following way: hybrid embryos were removed from fruit at 10-day intervals starting at 90 and going to 130 DAP (days after pollination). To achieve embryo isolation the fruit was washed with detergent and wiped with 70% (v/v) ethanol prior to being opened in a laminar airflow cabinet. The seed was removed and, for extra precaution, surface sterilized in a sodium hypochlorite solution (4%, v/v) for 5 minutes before being rinsed with sterile water tree times. Seed was then dissected and zygotic embryos removed and cultured on a medium containing 0.5 MS nutrients, sucrose (85 mM), NAA (0.25 µM) and kinetin (0.25 µM) or NAA (0.25 µM) and kinetin (1 µM). Two physical medium conditions were investigated for their suitability for embryo growth *viz*, solidified with agar (0.8% agar) or liquid, without agar. In the latter case cultures were shaken (90 rpm) on an orbital shaker.

The embryo cultures were then incubated under the standard culture conditions using a 16 h photoperiod at 25 $\pm$ 1^0 C for 2 weeks. Observations on percent germination, leaf area production, number of new leaves produced and the length of seeding hypocotyls were taken 2 weeks later. At this time the seedlings were divided randomly into two: one group was placed on a De Fossard plant growth regulator-free, liquid medium while the second group being placed on a MS, plant growth regulator-free, solid medium. The number of seedlings that were produced 2 weeks later were then counted and transferred to a nodal culture medium (Drew, 1988). The plants produced were then acclimatized.

Statistical Analysis

All experiments were undertaken using a complete randomized design with the number of replicates used varying between experiments. There were 5 replications per treatment for embryo rescue experiment (five petri dishes each with five zygotic embryos for solid culture, and 5 erlenmeyer bottles each with five zygotic embryos for liquid cultures).

RESULTS

Interspecific Hybridization

The first attempt at interspecific hybridization was carried out during a cool period, from the (October 1994) and into the summer (February 1995). This attempt produced few fruit (3%) containing just a few zygotic embryos (eight in total) (Table 1; first cross). Only one of these zygotic embryos developed sufficiently to allow somatic embryogenesis experiments to be undertaken. During this first hybridization study no attempt was made to undertake the reciprocal cross.

Table 1. Percentage of fruit set and percentage of seed containing zygotic embryos following interspecific hybridization attempt between papaya x *C. parviflora* (A. DC) Solms.

Crossing		Flowers pollinated	Fruit set (%)	Seed produced	Embryos produced
Seed parent	Pollen parent				
C. papaya	*C. parviflora*	150	5 (3.3)	50	8 (16)
C. papaya	*C. parviflora*	450	52 (11.5)	4500	1500 (33)
C. parviflora	*C. papaya*	25	0 (0)	0	0 (0)

The second interspecific hybridization attempt was carried out in a warmer period, from early summer (November 1995) to late summer (February 1996) (Table 1; second cross and reciprocal). During this second hybridization study the reciprocal cross was undertaken (Table 1; third cross).

Embryo Rescue of Hybrid

The zygotic embryos isolated from the seed following the crosses could be categorized as either being single or multiple (polyembryonic). The frequency of the polyembryony type was low (5%) and the size of these embryos varied considerably even in one seed. At the time of isolation of most single embryos they were well developed, already at the cotyledonary stage of development. However, the size of all embryos, these would already be at late cotyledonary stage of development at this time.

The ability of all hybrid zygotic embryos to germinate was in both liquid and on solid media. The germination was higher (Figure 1), leaves were larger (Figure 2), and more numerous (Figure 3) and hypocotyls longer (Figure 4) when a liquid medium was kinetin had no significant effect on the germination percentage but the seedlings produced on kinetin (1 µM) did produce greener leaves and were bigger than those prduced in the lower concentration of kinetin (0.25 µM; Figure 2). The germinated seedlings were then placed onto a De Fossard plant growth regulator0free medium or a MS plant growth regulator-free mediun. Five days later one third of those on the first medium the turned white, had become vitrified and died. Approximately one third formed seedlings with normal morphology (green leaves and stems, and healthy roots

These could be matured and were able to be propagated by a nodal culture technique (Drew, 1998), All plants were then acclimatized, and grown in pots in the greenhouse.

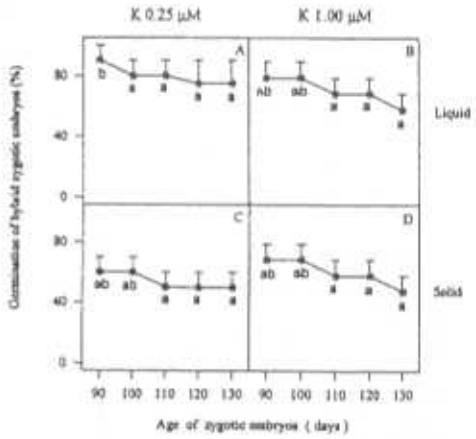

Figure 1. The effect of kinetin on the 2 weeks germination of papaya x *C. Parviflora* (A. DC.) Solms. hybrid embryos when placed on two different media 2 weeks affter initiation. Both media contained 0.5 Murashige and Skoog nutrients and NAA (0.25 µM), however one medium was liquid (A and B) while the other was solidified with 0.8% agar (C and D). Kinetin concentrations were 0.25 µM (A and C) and 1 µM (B and D). The results are the mean of 25 replicate cultures. Error bars are standard errors and points marked with the same letter are not significantly different.

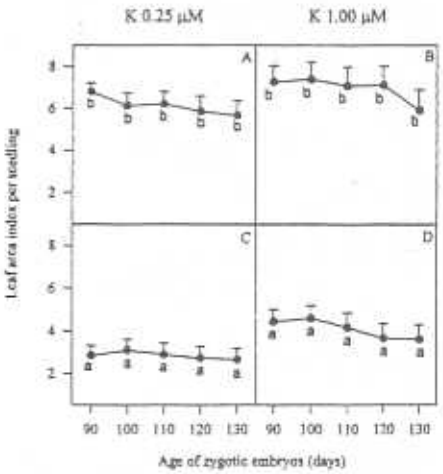

Figure 2. The effect of kinetin on the leaf area index of hybrid of papaya x C. Parviflora (A. DC.) Solms. on two different media. Both contained 0.5 Murashige and Skoog nutrients, NAA (0.25 µM), however one medium was liquid (A and B) while the other was solidified with 0.8% agar (C and D). Kinetin concentrations were 0.25 µM (A and C) and 1 µM (B and D). The results are the mean of 25 replicate cultures. Error bars represent the standard errors of the mean and points marked with the same letter are not significantly different.

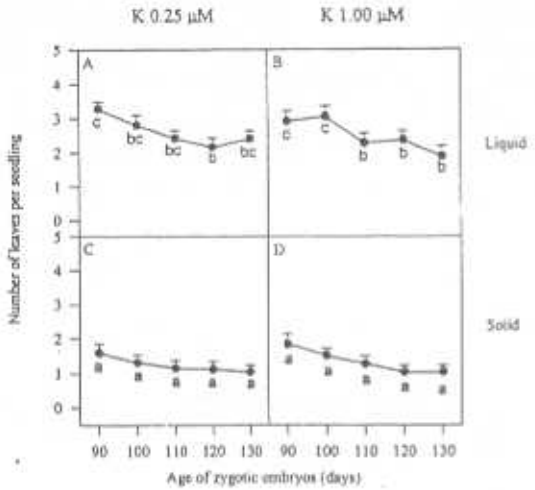

Figure 3. The effect of kinetin on the number of leaves of hybrid of *C. papaya* L. x *C. parviflora* (A. DC.) Solms. on two different media at 2 weeks after initiation. Both contained 0.5 Murashige and Skoog nutrients, NAA (0.25 µM), however one medium was liquid (A and B) while the other was solidified with 0.8% agar (C and D). Concentrations of kinetin were 0.25 µM (A and C) and 1 µM (B and D). The results are the mean of 25 replicate cultures. Error bars represent the standard error of the means and points marked with the same letter are not significantly different.

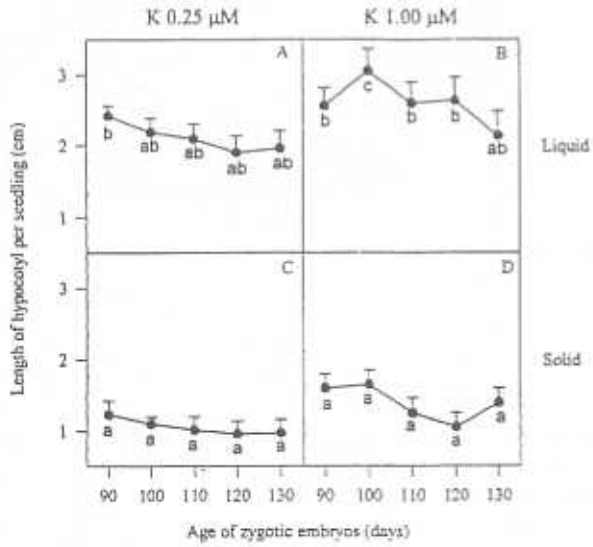

Figure 4. The effect of kinetin on the length of hypocotyl of hybrid of papaya x *C. parviflora* (A. DC.) Solms. on two different media at 2 weeks after initiation. Both contained 0.5 Murashige and Skoog nutrients, NAA (0.25 µM), however one medium was liquid (A and B) while the other was solidified with 0.8% agar (C and D). Kinetin concentrations were 0.25 µM (A and C) and 1 µM (B and D). The results are the mean of 25 replicate cultures. Error bars are standard errors and points marked with the same letter are not significantly different.

DISCUSSION

These studies used an already existing hybridization protocol (Magdalita *et al.* 1997) for papaya x *C. parviflora* (A. DC.) Solms. and the production of hybrid plants in soil. The successful production of hybrid was dependent on a number of factors which include the genotype used as the seed parent cross (papaya better than *C. parviflora*, Table 1) and the time of the year when the cross is to be undertaken (crosses better undertaken in the warmer months of the year, Table 1). In the case of successful embryo rescue, the hybrid embryos had to be isolated from the fruit that had reached the age of between 90 and 120 DAP. When rescued, a reasonable number of hybrid embryos survived and 60% of these germinated into normal plantlets with healthy root and shoot systems. An additional 20% produced normal shoots but did not have good root system and subsequently died. The remainder underwent somatic embryogenesis producing globular-the heart- then cotyledonary-shaped somatic embryo on the same media. Another 20% produced abnormal shoots, and callus which then differentiated into somatic embryos.

CONCLUSION

The best time for wide hybridization of papaya x *C. parviflora* (A. DC.) Solms. was from 8.00 am to 9.00 am in a warm summer period and using papaya as the seed parent. The best medium for germination of the hybrid zygotic embryos was 0.5 liquid MS medium containing sucrose (85 mM), kinetin (1 µM) and NAA (0.25 µM).

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