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Promotion of Immature Seed Germination in *Jacaranda mimosifolia*

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Abstract. **In vitro germination of immature seeds of *Jacaranda mimosifolia* treated with gibberellic acid (GA_3) was studied. Immature seeds were collected monthly after crossings and sown on Murashige and Skoog (1962) medium with 3.0% sucrose and 0.6% agar after soaked 24 hours with 0, 10, 100, and 500 mg·L^{-1} GA_3 solutions. Though germination was observed in the immature seeds harvested 2 months after crossing (2 MAC), the rate was quite low. When immature seeds of 3 MAC treated with 100 or 500 mg·L^{-1} GA_3 solution were cultured, >60% germination were obtained within 2 weeks after culturing. These results indicate that immature seeds of 3 MAC treated with adequate GA_3 solutions, seedlings can be obtained precociously and the period from crossing to the seedling stage was shorter than for mature seeds.**

Jacaranda mimosifolia D. Don, native to northwest Argentine and southern Brazil, is one of the most beautiful ornamental trees with attractive lilac-colored flowers (Gentry, 1992). Although this species is favorable for planting along avenue and parks, the utilization of this species is restricted to tropical and subtropical regions because of its poor tolerance to cold climate. Cold hardiness in *Jacaranda*, therefore, seems to be one of the most important objectives in the breeding of this plant. Since *Jacaranda* seeds require more than 6 months for maturation after pollination, the long period from crossing to obtaining of the seedlings constitute an obstacle in the cross breeding of this species.

Shortening the period from pollination to the seedling stage was attempted by ovule, ovary or embryo culture in some horticultural plants (Anderson et al., 1990; Torresán et al., 1996; Yasugi, 1984). To obtain seedlings through ovule culture effectively, plant growth regulators are often used (Sharma et al., 1996).

The objectives of this research were to study the effect of gibberellin on immature seeds germination *in vitro* and to establish a method to shorten the period from crossing to seedling stage in *Jacaranda mimosifolia*.

Materials and Methods

The intraspecific crossings among 11 genotypes of *Jacaranda mimosifolia* with 5-year-old grafted plants collected from various parts of Argentine were conducted in October 2002 in an unheated greenhouse of the Technological Center on Floriculture, Fruit Culture and Horticulture of Japan International Cooperation Agency (JICA) in Argentina (lat. 34° 36'S; long. 58° 40'E). Flowers for crossings were restricted to four or five in each flower cluster and fresh pollen was used for pollination.

Fruit were collected monthly until 5 months after crossing (MAC). The immature seeds were taken from the fruit that were disinfected by soaking in 70% ethanol for 5 min and were soaked with 0, 10, 100, and 500 mg·L^{-1} GA_3 solutions for 24 h. After GA_3 treatment, the immature seeds were immersed in 1.0% sodium hypochlorite for 30 min, rinsed three times with sterile distilled water and sown on 20 mL of culture medium under aseptic conditions. The culture medium was MS (Murashige and Skoog, 1962) with 3.0% sucrose and 0.6% agar in 200 mL culture bottles covered with aluminum foil and autoclaved at 121 °C for 20 min. Culture was incubated at 25 °C under 16 h light with cool-white fluorescent lamps yielding 25 μmol·m^{-2}·s^{-1} PPFD. Four replications using 18 seeds in each treatment were conducted in 1 to 3 MAC immature seeds whereas three replications in 4 and 5 MAC immature seeds. Number of germinated seeds was recorded weekly until 8 weeks after sowing.

Results and Discussion

Germination was not observed in 1 MAC immature seeds (Table 1). Though germination was observed in 2 MAC immature seeds, the rate was low. Immature seeds of 3 MAC showed 68.2% and 72.9% of germination rate after 100 or 500 mg·L^{-1} GA_3 treatment, respectively, whereas the rate was low in 10 mg·L^{-1} or without GA_3 treatment. There was no significant difference among treatments when 4 MAC immature seeds were used. Since the seeds of 5 MAC showed >80% of germination with and without GA_3 treatment, it seemed that they have already matured. Germination started one week after sowing and almost completed 2 weeks after sowing (Fig. 1). Almost all seedlings obtained from immature seeds survived after acclimatization and grew vigorously as shown in Fig. 2.

To obtain seedlings through ovule culture effectively, growth regulators are extensively used. Sharma and Gill (1982) reported that a low concentration of auxins is favorable for normal growth, whereas GA_3 is more or less effective in the enlargement of the wheat embryos. On the other hand, the effectiveness of GA_3 treatment for rapid germination of early harvested ovules was reported in *Azalea* (Michishita et al., 2001), *Malus* (Røen, 1994), *Helianthus* (Torresán et al., 1996). In this investigation, low germination was observed in 2 MAC immature seeds of *Jacaranda mimosifolia* as the result of GA_3 treatment. However, these seedlings could not grow normally and died before acclimatization (Fig. 2). The 3 MAC immature seeds seem to be the required maturity stage for GA_3 treatments to be effective.

Jacaranda mimosifolia has a long juvenile phase of about 4 to 5 years before flowering. Therefore, many years might be needed from the beginning of a breeding program to obtain a plant with desirable characteristics. It seems that this is the reason for the lack of reports, presently, on the release of new varieties by cross breeding in this species.

From our results, it was indicated that by exposing immature seeds of 3 MAC of *Jacaranda mimosifolia* to 100 or 500 mg·L^{-1} GA_3 for 24 h, hybrid seedlings can be obtained precociously and the breeding period of this species might be shortened by this technique.

Table 1. Effect of GA_3 treatment on germination of immature seeds obtained from intraspecific crossing of *Jacaranda mimosifolia*.

GA_3 (mg·L^{-1})	Germination (%)[z]				
	1 MAC	2 MAC	3 MAC	4 MAC	5 MAC
0	0.0 a[y]	6.4 a	7.3 a	38.5 a	80.3 a
10	0.0 a	13.9 a	20.8 a	50.4 a	82.4 a
100	0.0 a	6.3 a	68.2 b	69.0 a	95.8 a
500	0.0 a	6.9 a	72.9 b	77.0 a	93.8 a

[z]Data were collected after 1 month culture.

[y]Different letters in the same column indicate significant differences by Duncan's multiple range test at 5% level.

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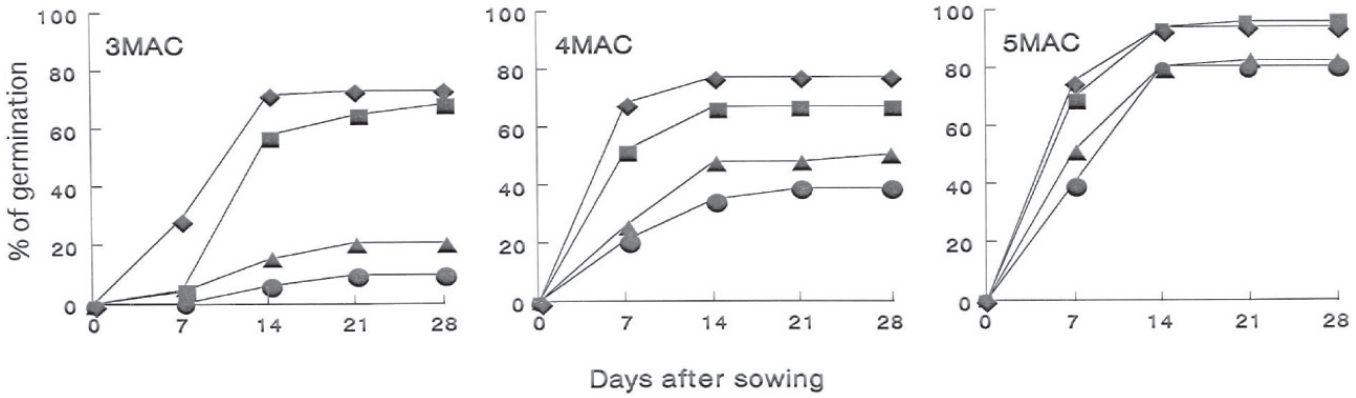

Fig. 1. Changes in germination rate of immature seeds of *Jacaranda mimosifolia* sown in vitro. GA_3 level ($mg \cdot L^{-1}$): 0 = ●, 10 = ▲, 100 = ■, 500 = ♦.

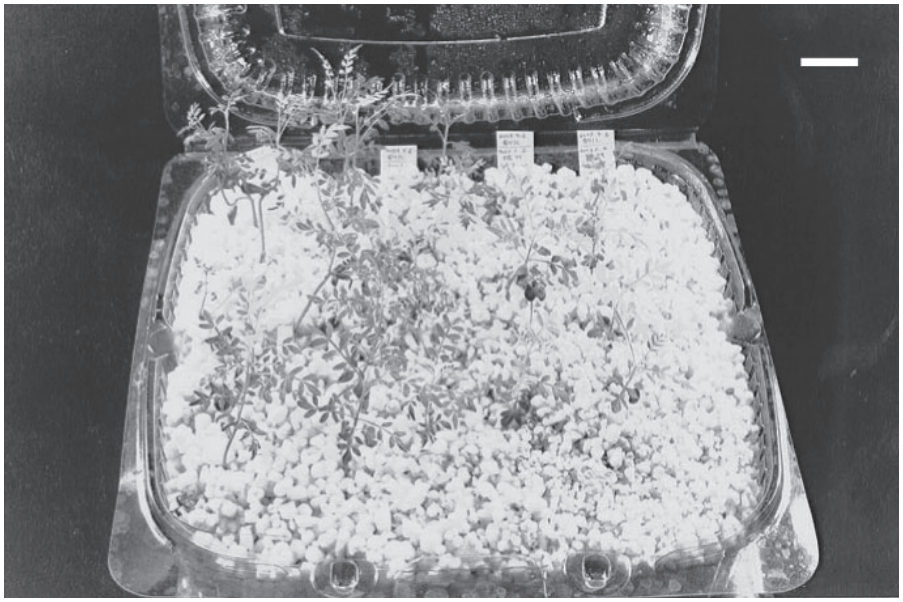

Fig. 2. Acclimatized plantlets obtained from 3 months after crossing (MAC) seeds of *Jacaranda mimosifolia*. Bar = 20 mm.

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