*Seed Science and Biotechnology* ©2008 Global Science Books

# *Ex Vitro* Performance of Peanut Plants from TDZ-pretreated Seeds

**Shweta Singh • M. M. Jana • Sulekha Hazra** *

Plant Tissue Culture Division, National Chemical Laboratory, Council of Scientific and Industrial Research, Pune, 411008, India

*Corresponding author*: * **s.hazra@ncl.res.in** or **sulekha@gmail.com**

## ABSTRACT

Thidiazuron (TDZ) is a potent regulator of morphogenetic responses in a large number of species. The activity of TDZ varies widely depending on its concentration, exposure time, explant and species. In the present experiment, the effect of TDZ on peanut (*Arachis hypogaea*) seed germination and plant growth was evaluated by soaking the mature seeds of cultivar SB-11 for 12 h at various concentrations of TDZ before sowing in a sand: soil mixture in pots and growing the plants for 17 weeks (crop duration of this cultivar is 110-115 days) in a greenhouse. Shoot and root elongation was reduced in the plants raised from TDZ-treated seeds. Elongation of the hypocotyl remained unaffected. Flowering was delayed in plants raised from TDZ pretreated seeds. Flowering was further delayed in the plants raised from seeds treated with higher concentrations of TDZ. Flowers and flower buds were noted at the cotyledonary node during harvesting of plants raised from seeds treated with 22.71 µM TDZ. The number of pods was optimum in plants developed from seeds treated with the lowest concentration (2.27 µM). There were no nodules on hypocotyls of TDZ-treated seed-derived plants. In the control, the hypocotyl was full of nodules. The effect of TDZ pretreatment has never been tested on peanut seed germination and plant growth. Retarded growth and delayed flowering indicated that the amount of TDZ absorbed by the seeds affected the plant through its entire life cycle.

**Keywords:** cultivar SB-11, *ex-vitro* plants, thidiazuron

## INTRODUCTION

Plant growth regulators (PGRs) are known to influence plant growth and development at very low concentrations (Jules *et. al.* 1981). Thidiazuron (TDZ), a substituted phenyl urea (Mok *et al.* 1982) has been established as a potent regulator of morphogenetic responses in a large number of species as well as diverse experimental systems. It induces as many or more adventitious shoots than adenine-type cytokinins in most species in which it has been tested (Lu 1993). It has a diversity of physiological effects on treated plants, which depends on concentration, time of application, plant species and cultivar. This in association with environmental conditions might have substantial impacts on the plant physiological response to the product (Amarante *et al.* 2002). In an experiment with apple trees two cvs., 'Gala' (characterized by having low fruit set) and 'Fuji' (characterized by having high fruit set), when sprayed with TDZ at different doses, increased shoot growth. Fruit set increased with TDZ dose in 'Gala' but not in 'Fuji'. It was concluded that TDZ sprayed at full bloom might improve fruit set in cultivars with deficient pollination (Amarante *et al.* 2002). In soybean (cvs. 'Pungsan' and 'Manlee'), spraying 2-(2,4-dichlorophenoxy) propanoic acid (2,4-DP) and benzyl amino purine (BAP) at early reproductive stages demonstrated that exogenous PGRs significantly influenced reproductive and growth characteristics, and consequently seed yield. With the exception of a low (0.5 mM) BAP treatment in cv. 'Pungsan', all treatments increased the number of pods with varying numbers of seeds per pod. Low 2,4-DP (0.04 mM) and BAP (0.5 mM) significantly reduced flower abortion (Cho *et al.* 2002) and delayed abscission of pods in both genotypes, resulting in increased pod setting. Foliar spray of some PGRs ($GA_3$, IAA (indole-3-acetic acid), BAP) on *Vicia faba* in a field trial led to significant changes in plant height, average number of leaves, leaf area per plant and dry weight of the shoot (Ibrahim *et al.* 2007). Application of BAP and IAA at 100 ppm caused a reduction in the percentage flower abscission and produced the highest number of pod setting. Spraying the foliage of lentil plants with kinetin (10, 20 and 40 mg/l) resulted in reduced stem height, an increase in the number of leaves, branches, shoot dry weight, number of produced flowers per plant, number and weight of pods and seeds per plant (Khalil *et al.* 2006). In peanut, foliar application of a low concentration (0.25 mg/l) of 28-homobrassinolide during flowering and pegging increased pod yield (Ramraj *et al.* 1997). The above studies were conducted on fully-grown plants and the PGRs were sprayed either before, during or after the plant reached the reproductive phase. However, there is no literature on growth and yield of a plant developed from seeds pretreated with PGRs.

The effects of TDZ on *in vitro* morphogenesis in peanut have been studied widely (Saxena *et al.* 1992; Murthy *et al.* 1995; Chengalrayan *et al.* 1997; Joshi *et al.* 2003). The activity of TDZ varied with concentration, exposure, explant and species (Murthy *et al.* 1998). In *in vitro* studies it was demonstrated (Joshi *et al.* 2003) that incorporation of TDZ into medium influences the meristem of somatic embryos resulting in the formation of plants with multiple shoots. Elongation of the shoots could be achieved on dilution of TDZ in the plantlets by repeated transfer to PGR-free medium. However the productivity of these plants with multiple shoots was never assessed. As somatic embryos are similar to the zygotic embryos, the present study was conducted to test the influence of TDZ on germination, branching pattern, flowering and pod yield in plants raised from TDZ-pretreated seeds having zygotic embryos.

## MATERIALS AND METHODS

Peanut (*Arachis hypogaea*) seeds of cv. 'SB-11' were procured from local market. Seeds were selected visually for uniformity. These were soaked for 12 h in aqueous solution of TDZ of concen-

Received: 26 May, 2008. Accepted: 23 June, 2008.

Original Research Paper

**Table 1** Germination and growth characteristics (all values mean ± SD) of peanut plants raised from TDZ-pretreated seeds.

| TDZ (µM) | Germination (%) | Shoot length* (cm) | Hypocotyl length (cm) | Root length (cm) | № of branches | Total № of pods | № of seeds per plant | Seed wt per plant (g) |
|---|---|---|---|---|---|---|---|---|
| Control | 38 ± 8.4 | 12.4 ± 1.6 a (18) | 3.4 ± 1.3 | 15.7 ± 2.0 a | 5.7 ± 0.7 | 13.1 ± 3.8 ab | 20.4 ± 9.5 a | 3.3 ± 1.5 a |
| 2.27 | 38 ± 16.4 | 9.3 ± 0.4 b (18) | 4.1 ± 0.9 | 9.9 ± 1.5 b | 5.5 ± 0.6 | 15.7 ± 4.4 a | 17.1 ± 3.7 ab | 2.4 ± 0.3 ab |
| 4.54 | 30 ± 14.1 | 8.9 ± 2.0 bc (15) | 3.8 ± 0.9 | 8.8 ± 1.8 bc | 5.3 ± 1.1 | 10.4 ± 3.3 ab | 16.7 ± 4.5 abc | 2.4 ± 0.5 abc |
| 9.08 | 36 ± 13.4 | 8.1 ± 0.9 bcd (16) | 4.4 ± 0.9 | 8.1 ± 0.9 bc | 4.9 ± 1.0 | 11.7 ± 3.5 ab | 13.2 ± 2.9 abc | 2.1 ± 1.1 abc |
| 22.71 | 24 ± 21.9 | 6.2 ± 1.6 d (12) | 4.0 ± 1.5 | 5.2 ± 1.9 d | 4.3 ± 0.9 | 6.0 ± 5.8 b | 6.7 ± 7.1 c | 0.2 ± 0.3 d |
| ANOVA | ns | $P<0.01$ | ns | $P<0.01$ | ns | $P<0.05$ | $P<0.05$ | $P<0.05$ |

[a-d] Duncan multiple range notation. Means followed by the same superscripts within a column do not differ significantly at P≤0.05.
*Figures in parenthesis indicate number of plants

trations 2.27, 4.54, 9.08 and 22.71 µM. Seeds soaked in distilled water without any PGRs were treated as control. Soaked seeds with adhering TDZ were planted in sand soil mixture (1:1) in earthen pots 22 cm in diameter and 18 cm in height. Plants were grown in green house at 25/18°C (day/night temperature) under natural day length.

The normal crop duration (from germination to harvesting) of 'SB-11' is 110-115 days. Till flowering the plants were identified as seedlings. The morphological characters like shoot, root and hypocotyl length, number of branches per plant and pod yield were noted when plants were harvested after 17 weeks (119 days). The main shoot was measured for shoot length. The length of the hypocotyl was determined by measuring the length between the remainder of the cotyledon and the point from where the root started. Root length was scored by measuring the distance from the end of the hypocotyl to the tip of the main root. Plant yield was scored by counting the pods, number of seeds from each plant and by weighing the seeds/plant.

The experiment was repeated five times with 10 seeds for each treatment. However, all seeds did not germinate and a few were lost before harvesting. Therefore the data was collected from the surviving plants (**Table 1**) after 119 days. All data were subjected to analysis of variance (ANOVA). Data were analyzed by one-way ANOVA and the means were compared by Fisher's LSD test at P≤0.05. Values are the means of five independent experiments. The differences among the treatment means were tested using Duncan's multiple range test (DMRT) at the 5% probability level (P<0.05).

## RESULTS AND DISCUSSION

This is the first report on effect of TDZ on peanut seed germination and plant growth in soil. Plant growth regulators including BAP (Ibrahim *et al.* 2007), kinetin (Khalil *et al.* 2006), homobrassinolide (Ramraj *et al.* 1997), TDZ (Amarante *et al.* 2002), among others, have been tested to increase growth/yield in crops. However TDZ have never been tested to increase the growth/yield of peanut. Secondly, the majority of PGRs are used as a foliar spray in mature plants to test the effect on crop yield. Keeping in view the persistence of TDZ in peanut tissues even after removal of the PGR in *in vitro* studies (Joshi *et al.* 2003), peanut seeds were soaked in TDZ solution and the coating of TDZ on the seed surface was retained during planting of the seeds.

Germination started in the seeds without TDZ treatment in 5-8 days and displayed normal seedling development. The germination period of seeds was similar to the control at 2.27, 4.54, and 9.08 µM TDZ and was delayed by 7-10 days in seeds treated with 22.7 µM TDZ. However, the frequency of germination (**Table 1**) was not significantly affected. In seeds of large-seeded grain legumes (*Phaseolus acutifolius*, *P. aureus*), cytokinins did not affect seed germination (Malik and Saxena 1992).

The periods taken by the seedling to develop into mature plant to flower differed in TDZ-treated seed derived seedlings and in the control. Flowering started in control plants in 4-6 weeks from date of planting. After 8 weeks, the plant length reached their optimum (**Fig. 1A**) and thereafter, the vegetative growth in control was retarded and the reproductive phase (flowering and pegging) was enhanced. Flowering was delayed in plants grown from seeds treated with TDZ (**Table 2**). In 2.27 µM TDZ treated seed derived plants, flowering was delayed by only 2-3 days but in 4.54 µM and 9.08 µM of TDZ it was delayed by 7-10 days and in 22.71 µM of TDZ it was delayed by 14-20 days. After 17 weeks in pots (**Fig. 1B**), plants were harvested. The optimum shoot length in control plants was 12.4 cm (**Table 1**). As the concentration of TDZ increased, the shoot length decreased (**Fig. 1A, 1B**); it was minimum (6.2 cm) in plants obtained from 22.71 µM TDZ-treated seeds (**Fig. 1D**). In seedlings raised from 22.71 µM TDZ-treated seed, initially the leaves were wrinkled and small (**Fig. 1C**) after germination but on maturity, there was no difference in leaf morphology. On measuring the hypocotyl and roots in the harvested plants it was observed that TDZ not only retarded shoot length but also influenced root length and growth of rootlets (**Fig. 1D**). Root length decreased as the concentration of TDZ increased and shortest roots (5.2 cm) were noted in plants derived from seeds treated with 22.71 µM of TDZ. Roots were longest in the control at 15.7 cm (**Table 1**). In contrast to shoots and roots, TDZ did not have any effect on the elongation of hypocotyls. An *in vitro* experiment with lotus (Nikolic *et al.* 2006) reported that TDZ retarded shoot length up to 1/3$^{rd}$ of the control even at lower concentrations of TDZ whereas at higher concentrations, the inhibition was stronger. In the present experiment, we observed that TDZ retarded both shoot and root elongation as the concentration of TDZ increased (**Table 1**).

The effects of cytokinins on seed germination, elongation of seedling shoots and roots, the frequency of regeneration, and the number of regenerants (somatic embryos or shoot bud) per seedling were determined in *Lotus corniculatus* in an *in vitro* experiment (Nikolic *et al.* 2006). They reported that not all parts of shoots were equally affected. The hypocotyls were not inhibited by cytokinins. In the present experiment we noted that there was no significant difference in hypocotyl length (**Table 1**) between control and plants raised from TDZ-treated seed. Root nodules were present in control as well as in plants raised from TDZ-treated seed; interestingly there were no sign of nodules on the hypocotyls of plants raised from TDZ-treated seed whereas in controls, the hypocotyl was full of nodules (**Fig. 1E**). In leguminous plants, nodules are formed on roots in response to infection with *Rhizobium leguminosarum*. The absence of nodules in hypocotyls of plants raised from TDZ-treated seed indicates the lack of infection in these plants.

There were no significant difference in the number of branches per plant between control and plants raised from TDZ-treated seed (**Table 1**). Multiplication of shoots of pongamia (Sujatha and Hazra 2006), and pigeonpea (Singh *et al.* 2003) in the presence of TDZ *in vitro* was demonstrated earlier. However, in these studies the tissues were continuously exposed to the PGR.

In contrast to somatic embryo-derived plants developed in the presence of TDZ, there was no multiple shoot development in plants grown from seeds treated with TDZ (**Table 1**). As flowering was delayed in plants raised from seeds pretreated with 22.71 µM of TDZ, flower and flower buds were present (**Fig. 1F**) at the cotyledonary node of these plants at the time of harvest.

Among all TDZ treatments, the number of pods per plant was maximum when seeds were treated with 2.27 µM of TDZ. This was followed by the control plants. It was lower than the control in other concentrations of TDZ, and

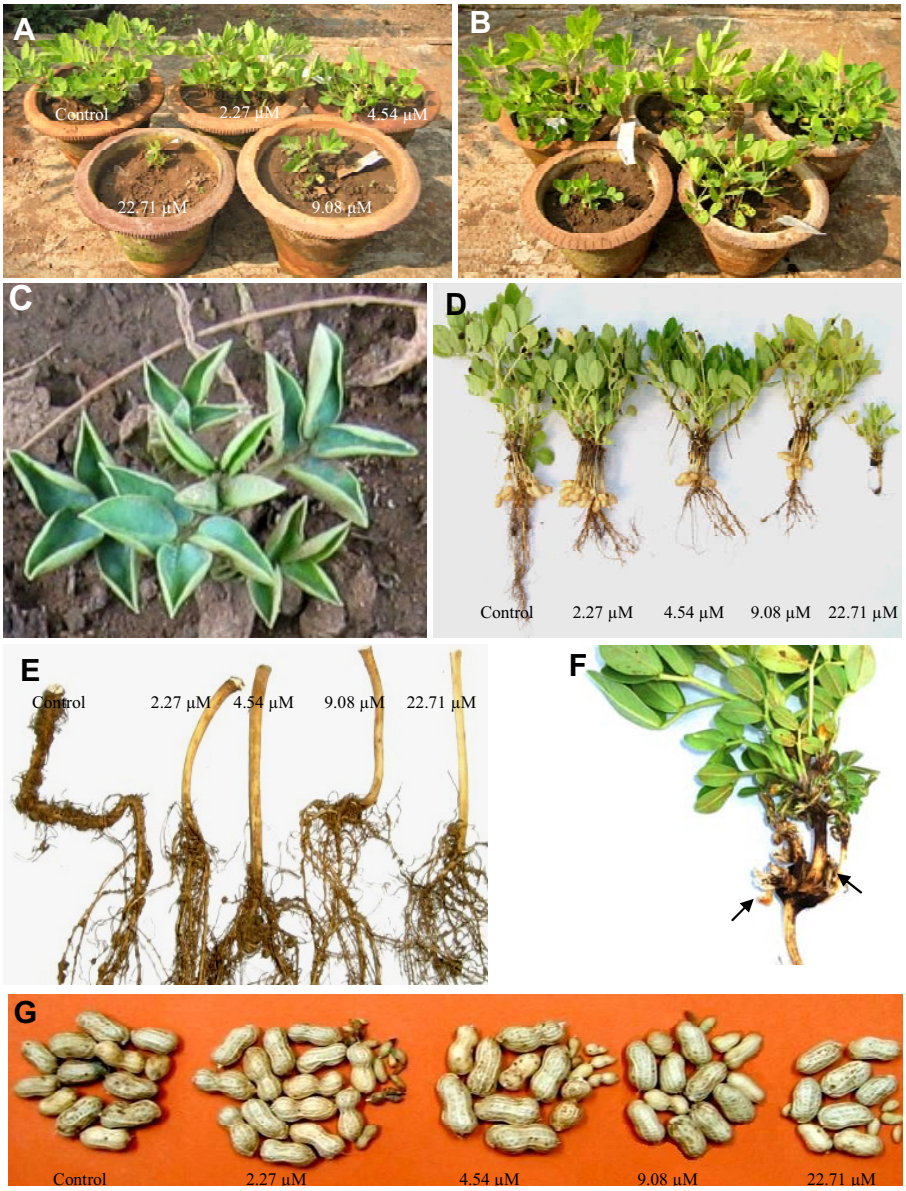


**Fig. 1 Effect of TDZ on peanut plants raised from TDZ-pretreated seeds.** (**A**) Plants after 8 weeks of planting. (**B**) Plants after 17 weeks of planting (before harvesting). (**C**) Seedling with wrinkled leaf raised from seed treated with 22.71 µM of TDZ. (**D**) Plants after harvesting. Plants raised from seeds treated with TDZ (2.27-22.71 µM of TDZ) were shorter in length. (**E**) Roots on harvesting. Nodule formation was restricted to the hypocotyls of the plants raised form TDZ-treated seeds. (**F**) Plant grown from seeds pre-treated with 22.71 µM of TDZ, showing flowering from the cotyledon nodes (black arrow) at the time of harvesting. The pods were removed earlier. (**G**) Pods obtained from the plants raised from seeds pretreated with TDZ and control seeds. Total number of pods was higher in plants raised from seeds treated with 2.27 µM of TDZ. Many of the pods were immature and had a soft shell.

was minimum at 22.71 µM of TDZ (**Table 1**). During harvesting, almost all pods obtained from control plants had a hard shell. However, some of the pods on plants derived from TDZ-treated seed were still soft (**Fig. 1G**) indicating the immaturity of the pods. As the concentration of TDZ increased, so too did the number of immature pods. There was no obvious difference in pod shape compared to control (**Fig. 1G**). The soft-shelled pods were smaller in size and had immature seeds at various stages of development. The number of seeds and seed weight per plant were optimum in the control (**Table 1**). The number of seeds decreased as the concentration of TDZ increased. Late flowering (**Table 2**), in plants raised from TDZ-treated seed, resulted in delayed seed set. As all plants were harvested at the same time, the number of small pods with soft shell and undeveloped seeds were more in plants which had delayed flowering. Some of the seeds in the pods were too minute and were not scored as seed. This resulted in reduction in seed number and seed weight (**Table 1**). Although the number of pods was higher in plants developed from seeds treated with 2.27 µM TDZ, the total number of seeds per plant was less. This was due to the fact that some of the pods collected from these plants were immature and did not bear seed while others were soft-shelled and with immature seeds. It needs to be tested if on extended incubation the immature pods would mature to produce more seeds in pots in the greenhouse giving a higher yield by weight. However, that would increase the crop duration. TDZ has never been tested for increase in peanut crop yield. In the *in vitro* studies (Chengalrayan *et al.*

**Table 2** Delayed flowering in TDZ pre-treated seed derived peanut plants in comparison to control plants.

| TDZ (µM) | 2.27 | 4.54 | 9.08 | 22.71 |
|---|---|---|---|---|
| Delayed flowering (days) | 2-3 | 7-10 | 7-10 | 14-20 |

1997; Joshi *et al.* 2003), exposure of somatic embryos to medium containing TDZ gave rise to plants with multiple shoot primordia, a typical reaction of many plant species *in vitro* to TDZ. Differentiation of these shoot primordia to shoots could be achieved by repeated transfers to PGR-free medium. It was hypothesized that TDZ influenced the meristematic cells (Joshi *et al.* 2003) to proliferate and produce multiple buds and that it persisted in the plants causing retarded elongation of the shoots. Retarded elongation in TDZ-induced shoots has been reported in peanut (Chengalrayan *et al.* 1997; Joshi *et al.* 2003) and in tamarind (Mehta *et al*. 2004). In the present experiment, seeds were exposed in TDZ for only 12 h prior to sowing. The amount of TDZ absorbed and adsorbed by the seeds influenced the plant through out the life cycle of 119 days.

## ACKNOWLEDGEMENTS

We acknowledge CSIR, India for a Research fellowship awarded to Shweta Singh. We thank Dr. Mrs. Urmil J Mehta for her co-operation and support.

## REFERENCES

**Amarante CVT, Ernani PR, Blum LEB, Megguer CA** (2002) Thidiazuron effects on shoot growth, return bloom, fruit set and nutrition of apples. *Pesquisa Agropecuária Brasileira* **37**, 10

**Chengalrayan K, Mhaske VB, Hazra S** (1997) High-frequency conversion of peanut somatic embryos. *Plant Cell Reports* **16**, 783-786

**Cho Y, Suh SK, Park HK, Wood A** (2002) Impact of 2,4-DP and BAP upon pod set and seed yield in soybean treated at reproductive stages. *Plant Growth Regulation* **36**, 215-221

**Ibrahim ME, Bekheta MA, El-Moursi A, Gaafar NA** (2007) Improvement of growth and seed yield quality of *Vicia faba* L. plants as affected by application of some bioregulators. *Australian Journal of Basic and Applied Sciences* **1**, 657-666

**Joshi MV, Sahasrabudhe NA, Hazra S** (2003) Responses of peanut somatic embryos to thidiazuron. *Biologia Plantarum* **46**, 187-192

**Jules J, Rober WS, Frank WN, Varnon WR** (1981) *Plant Science. An Introduction to World Crops*, W. H. Freeman and Co. New York, pp 55-64, 162-192

**Khalil S, El-saeid HM, Shalaby M** (2006) The role of kinetin in flower abscission and yield of lentil plant. *Journal of Applied Science Research* **2**, 587-591

**Lu C** (1993) The use of thidiazuron in tissue culture. *In Vitro Cellular and Developmental Biology – Plant* **29**, 92-96

**Malik KA, Saxena PK** (1992) Somatic embryogenesis and shoot regeneration from intact seedlings of *Phaseolus acutifolius* A., *P. aureus* (L.) Wilczek, *P. coccineus* L., and *P. wrightii* L. *Plant Cell Reports* **11**, 163-168

**Mehta UJ, Barreto SM, Hazra S** (2004) Effect of thidiazuron in germinating tamarind seedlings. *In Vitro Cellular and Developmental Biology – Plant* **40**, 279-283

**Mok MC, Mok DWS, Armstrong DJ, Shudo K, Isogai Y, Okamoto T** (1982) Cytokinin activity of N-phenyl-N′-1,2,3-thiadiazol-5-ylurea (thidiazuron). *Phytochemistry* **21**, 1509-1511

**Murthy BNS, Murch SJ, Saxena PK** (1995) Thidiazuron induced somatic embryogenesis in intact seedlings of peanut (*Arachis hypogaea*): endogenous growth regulator levels and significance of cotyledons. *Physiologia Plantarum* **94**, 268-276

**Murthy BNS, Murch SJ, Saxena PK** (1998) Thidiazuron: a potent regulator of *in vitro* plant morphogenesis. *In Vitro Cellular and Developmental Biology – Plant* **34**, 267-272

**Nikolic R, Mitic N, Miletic R, Neskovic M** (2006) Effects of cytokinins on *in vitro* seed germination and early seedling morphogenesis in *Lotus corniculatus* L. *Journal of Plant Growth Regulation* **25**, 187-194

**Ramraj VM, Vyas BN, Godrej NB, Mistry KB, Swami BN, Singh N** (1997) Effects of 28-homobrassinolide on yield of wheat, rice, groundnut, mustard, potato and cotton. *Journal of Agricultural Science* **128**, 405-413

**Saxena PK, Malik KA, Gill R** (1992) Induction by thidiazuron of somatic embryogenesis in intact seedlings of peanut. *Planta* **187**, 421-424

**Singh ND, Sahoo L, Sarin NB, Jaiwal PK** (2003) The effect of TDZ on organogenesis and somatic embryogenesis in pigeonpea (*Cajanus cajan* L. Millsp). *Plant Science* **164**, 341-347

**Sujatha K, Hazra S** (2006) *In vitro* regeneration of *Pongamia pinnata* Pierre. *Korean Journal of Plant Biotechnology* **33**, 263-270