Influence of Pre-sowing Treatments, Sowing Positions and Age of Stones after Extraction from Fruit on Germination and Vigour of Mango

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ABSTRACT

Background: Mango stones are in general recalcitrant. Moreover, they are available only during April- May months which are the drier parts of the year. So stone germination and plant vigour are very low. Proper seed orientation, age of stones after extraction from fruit and pre-sowing treatments need to be taken into consideration for inducing early germination, boosting growth, enhancing the seedling vigour and reducing the mortality.

Methods: The experiment was laid out in factorial completely randomized design with forty two treatment combinations replicated thrice. The treatments were comprised of two sowing positions *viz.,* flat and stalk end up, three age of stones after extraction from fruit, *viz.*, fresh extracted, 10 days and 20 days after extraction and seven pre-sowing treatments *viz.,* 100 ppm GA_3, 200 ppm GA_3, 1 ppm KNO_3, 2 ppm KNO_3, cow dung slurry, water, control and their combinations.

Result: Stalk end up sowing of freshly extracted stones pre-treated with 200 ppm GA_3 for 24 hours recorded better germination and vigour of mango seedlings.

Key words: Age, Germination, Pre-sowing treatments, Sowing positions, Vigour.

INTRODUCTION

Being a recalcitrant seed, the viability of mango stone is comparatively low. There is only about 12-50 per cent germination when sown within a month after extraction (Gill *et al*., 1985). The availability of fruits are confined to mainly one season. So the stones which are available during a particular season need to be properly utilized and exploited in an effective way for raising strong, healthy and actively growing rootstocks.

Usually the mango stones are available during April-May months *i.e.* drier part of the year. Therefore the stone germination and plant vigour are critically very low. In our country, mainly non-descriptive monoembryonic seedlings are utilized for rootstock purpose (Patel *et al.,* 2016). Hence there is a great variation in stone germination, vigour and further seedling development depending on the location and region, where the rootstocks are raised.

Generally the stones begin to germinate 12 to 15 days after sowing, but may take about a month or even more to complete the germination. The sporadic and slow germination in mango is due to the stony endocarp and consequently seedlings take more time to attain graftable size. It is necessary to improve stone germination and enhance seedling growth for synchronization, rapid seedling emergence and healthy rootstocks within a short period of time (Patel *et al*., 2017). To achieve a perceptible difference in enhancing germination, rapid emergence, boosting up of growth and reducing mortality, sowing positions (seed orientation), age of stone after extraction from fruit and various pre-sowing treatments need to be taken into consideration.

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An experiment was therefore undertaken to assess the effect of pre-sowing treatments, sowing positions and age of stones after extraction from the fruit with the main objectives of increasing germination percentage and producing vigorous rootstocks in order to meet the ever rising market demand and to evolve the best technology for producing high quality mango planting material in a short period of time.

MATERIALS AND METHODS

The present study was carried out during 2018 - 2019 at the College of Agriculture, Vellayani, Thiruvananthapuram, Kerala. The experiment was laid out in factorial completely randomized block design with 42 treatments. The treatments comprise of different combinations of 2 sowing positions (flat and stalk end up), 3 different age group of stones after extraction from fruit (freshly extracted stone, 10 days after extraction and 20 days after extraction) and 7 pre-sowing treatments (GA_3- 100, GA_3 200 ppm, KNO_3-1 ppm, KNO_3-2 ppm, cow dung slurry, water and control). Fruits of 'Kotookonam Varikka' variety of mango were selected for the study. The average stone weight of each age groups were recorded accordingly. The average stone weight was 29.85 g for freshly harvested, 28.02 g for stones which were sown 10 days after extraction and 24.63 g for the stones which were sown 20 days after extraction respectively. After extraction, the stones were washed thoroughly and were soaked in various pre-soaking solutions for 24 hours prior to sowing. The pre-treated mango stones of different age groups were sown at proper spacing in stalk end up and flat positions in the seed bed. The germination of stones started 15 days after sowing and continued upto 55 days. Observations were recorded daily for germination parameters and vegetative parameters like seedling length, dry weight and seedling vigour index I and II were recorded 4 months after sowing.

The germination percentage was calculated as follows;

Germination percentage =

$$\frac{\text{Number of stones germinated}}{\text{Total number of stones sown}} \times 100$$

(Patel *et al*., 2016)

The rate of germination was calculated as;

Rate of germination =

$$\frac{\text{Germination percentage}}{\text{Number of days taken for germination}}$$

(Bewley and Black, 1982)

Vigour index - I (growth basis) and Vigour index - II (weight basis) were calculated using the formula given by Kumar *et al.* (2007) as follows;

Vigour Index –I = Germination percentage (%) × [Shoot length (cm) + Root length (cm)]

Vigour Index – II = Germination percentage (%) × Dry weight of seedling (g)

The experimental data recorded was subjected to statistical analysis as per the method suggested by Panse and Sukhatme (1967). Treatment means were separated using F test values at 5% level of significance.

RESULTS AND DISCUSSION

Significant differences were observed among the pre-sowing treatments, sowing position and age of stone after extraction from the fruit for germination characters. Statistically analysed results are given in Table 1 and 2 and are explained under following subheadings.

Effect of sowing positions

The perusal of data presented in Table 1 clearly indicated that the earliness in germination (22.95 days), the least number of days taken for 50 per cent germination (31.75 days), higher germination percentage (60.85%) and germination rate (0.47) were in stalk end up position as compared to flat method of sowing.

The stones sown in stalk end up position produced significantly higher seedling length (34.63 cm), the highest seedling dry weight (9.77 g) and produced the seedlings with higher vigour index-I (2176.50) and vigour index- II (603.27) on growth and weight basis (Fig 1 and Fig 2) accordingly than flat position.

The amount of energy required to accomplish germination varies according to genotype and seed orientation on seed bed because of the quantity of stored nutrients, especially endosperm and positioning of micropyle, respectively in cashew (Hammed *et al.,* 2014). Naturally, the radicle has a positive geotropism whereas the shoot of the germinating seed has a negative geotropism. Supporting the upcoming response of the seedling to the stimulus (gravity) is highly correlated with the orientation of micropyle. The tip of the root bends downward if the seeds oriented vertically upward with respect to micropyle. The roots of seed sown by mycropyle in vertically upright position need to curve over the seed itself in order to grow in downward (normal) direction in tree species (Coutts, 1989).

Mango stones with stalk-end up position of sowing places the micropyle in the most suitable position, *i.e.*, pointing downward, the roots of the seed grew easily and directly downward (does not require bending), which requires less energy for germination and radicle emerge from the embryo (Vijaya and Satyanarayana, 2004). Hence it resulted with highest germination percentage and earliness in germination in Stalk end up method.

The seedlings whose roots grow properly without any curvature will establish well for its function and growth, which can ultimately improve the performance. Hence, stalk end up method resulted in the highest seedling length and dry weight which ultimately resulted in better seedling vigour indices, both on growth and weight basis over the flat method of sowing.

The improper orientation of seeds could impoverish the emerging embryo for needed quantum of oxygen which could lead to the synthesis of higher amount of ethanol and pyruvate in the plant system and finally leads to the death of the emerging embryo (Bewley, 1997). This might be the probable reason for reduced germination and poor quantitative plant vigour in seeds sown in flat method.

Effect of age of stones after extraction from the fruit

The highest germination percentage, earliness in germination, rate of germination, higher seedling length, seedling dry weight, seedling vigour indices on growth basis and weight basis were the best for freshly harvested stones (Table 1).

The germination ability of a seed was directly related to its moisture content as well as the rate at which seeds lose its moisture thereby affecting the viability in *Calamus species* (Patil and Krishna, 2016). The reduction in viability

Table 1: Germination characteristics of mango stones as influenced by various sowing positions, age of stones after extraction from the fruit and pre-sowing treatments.

Parameters	Days taken for initiation of germination	Days taken for 50% germination	Germination (%)	Rate of germination	Seedling length (cm)	Dry weight of seedling (g)
Effect of sowing positions						
Flat (S_1)	29.15	40.91	40.95	0.26	28.12	7.23
Stalk end up (S_1)	22.95	31.75	60.85	0.47	34.63	9.77
SE (m)	0.05	0.17	0.69	0.001	0.10	0.04
CD at 5%	0.13	0.47	1.94	0.004	0.28	0.12
Effect of age of stone after extraction from fruit						
Freshly extracted stone (A_1)	18.56	31.29	59.84	0.47	36.60	9.85
10 days after extraction (A_2)	24.56	36.50	52.38	0.36	31.57	8.32
20 days after extraction (A_3)	35.03	41.20	40.48	0.28	25.95	7.33
SE(m)	0.06	0.20	0.85	0.002	0.12	0.05
CD at 5%	0.16	0.57	2.38	0.005	0.34	0.15
Pre sowing treatments						
GA_3 - 100 ppm (T_1)	23.89	33.94	55.19	0.43	35.70	10.39
GA_3 - 200 ppm (T_2)	22.62	31.78	62.59	0.48	34.70	10.01
KNO_3 - 1 ppm (T_3)	24.49	34.17	52.96	0.42	33.26	9.22
KNO_3 - 2 ppm (T_4)	25.69	35.56	50.00	0.36	32.27	8.59
Cow dung slurry (T_5)	25.78	35.78	53.19	0.35	30.05	7.77
Water (T_6)	28.84	40.11	42.96	0.31	27.82	7.12
Control (no treatment) (T_7)	31.01	42.94	37.41	0.25	25.83	6.39
SE (m)	0.09	0.31	1.29	0.003	0.19	0.08
CD at 5%	0.24	0.87	3.63	0.008	0.53	0.22

and vigour were proportional to increased leaching of metabolites from seeds and decreased dehydrogenated activity of seeds. The leaching of metabolites increases with decreased seed moisture content during storage.

The freshly extracted seeds had considerable amount of post imbibition hydrolysis of non-reducing sugars and DNA-P (DNA polymerase) which resulting in initiation of protein synthesis, little or none could be observed as age advances in mango (Chandra, 1980). The higher expression of DNA-P in imbibed seeds enhancing the protection against DNA damage and allows successful germination of rice seeds (Sihi *et al*., 2015). Germination became progressively slower as the age advanced.

Effect of pre-sowing treatments

It is evident from Table 1 that the pre-sowing treatments had a significant effect on growth and vigour of mango seedlings. The stones treated with 200 ppm GA_3 recorded the least number of days for initiation of germination (22.62 days), for 50 per cent germination (31.78 days), the highest germination percentage (62.59%) and rate of germination (0.48), significantly higher vigour index-I (2310.02) and vigour index-II (657.09) on growth and weight basis (Fig 1 and Fig 2). The highest seedling length (35.70 cm) and dry weight (10.39 g) of the mango seedlings were observed in 100 ppm GA_3. The poorest germination, vigour and growth characteristics of mango seedlings were observed in control (without any treatment).

The earliest stone germination in GA_3 might be due to the increased concentration of endogenous auxin content due to the GA_3 application as the GA_3 is the vital component of auxin signalling pathway (Ross *et al.,* 2002). In aonla, the increased level of auxin and enhanced enzymatic activities along with the repression of inhibitors might be the probable reasons for faster germination. GA_3 might have also triggered the starch hydrolysis and their translocation to the growing seedlings thereby inducing early germination (Rajmanickam *et al.,* 2004).

The presence of GA_3 inside the seed which stimulates the imbibition process on subsequent seed germination. Pre-soaking treatment of GA_3 might have affected directly and altered various enzymatic reactions, synthesis of proteins and conversion of starch into sugars involved in the process of germination (Paleg, 1960). On the other hand, GA_3 also

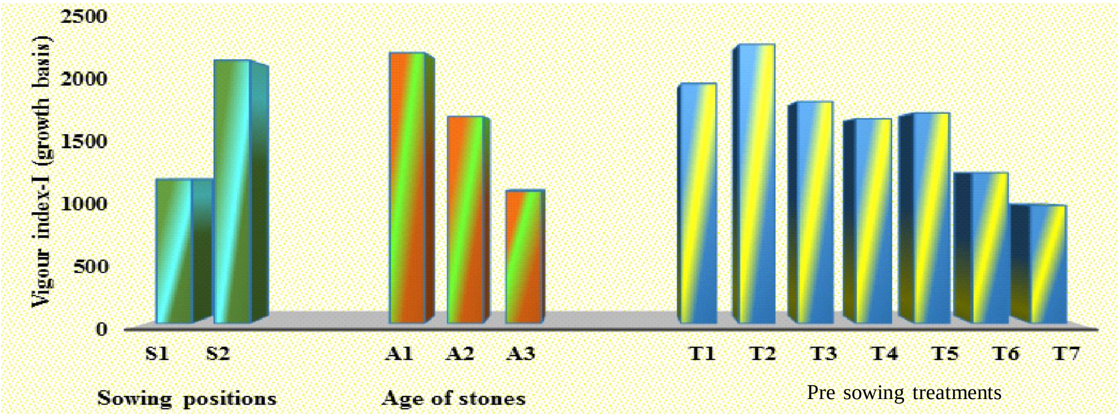

Fig 1: Effect of sowing positions, age of stones after extraction from the fruit and pre sowing treatments on vigour index -I (growth basis) of mango seedlings.

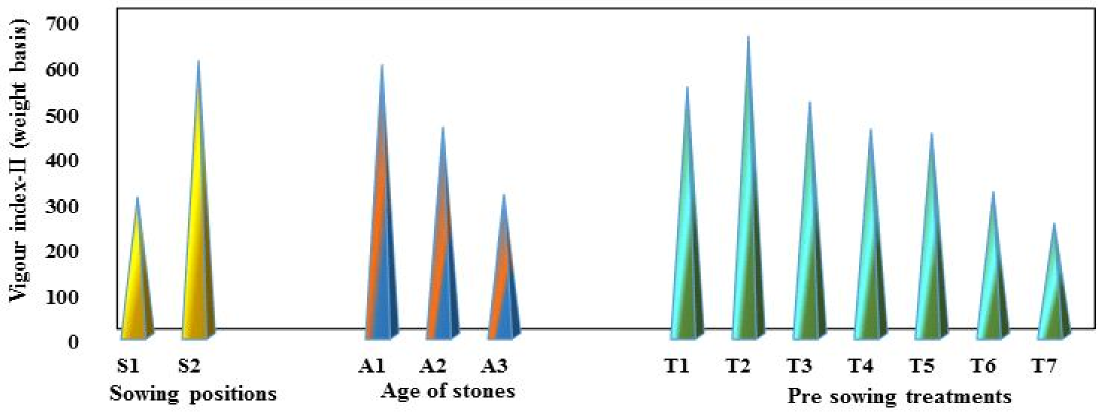

Fig 2: Effect of sowing positions, age of stones after extraction from the fruit and pre sowing treatments on vigour index –II (weight basis) of mango seedlings.

Table 2: Interaction effect of sowing positions, age of stones after extraction from the fruit and pre-sowing treatments on germination of mango stones.

Treatments	Days taken for initiation of germination	Days taken for 50% germination	Germination (%)	Rate of germination	Seedling length (cm)	Dry weight of seedling (g)	Seedling vigour index -I (Growth basis)	Seedling vigour index- II (Weight basis)
$S_1A_1T_1$	19.13	31.33	53.33	0.51	37.26	9.60	1918.80	511.44
$S_1A_1T_2$	17.73	30.33	62.22	0.58	36.02	9.16	2319.70	569.87
$S_1A_1T_3$	18.33	31.67	51.11	0.51	34.83	8.65	1781.80	442.24
$S_1A_1T_4$	21.00	33.67	46.67	0.40	34.42	8.36	1611.42	390.17
$S_1A_1T_5$	22.20	33.33	53.33	0.41	31.55	7.75	1680.84	412.84
$S_1A_1T_6$	23.73	38.34	40.00	0.26	29.50	7.35	1180.86	293.35
$S_1A_1T_7$	25.73	42.33	35.55	0.21	27.71	6.49	985.58	231.59
$S_1A_2T_1$	26.00	37.34	51.11	0.24	32.31	8.00	1348.99	351.10
$S_1A_2T_2$	24.00	36.33	42.22	0.25	31.92	8.32	1650.36	408.90
$S_1A_2T_3$	26.53	41.33	40.00	0.24	30.68	7.56	1230.04	302.38
$S_1A_2T_4$	27.67	41.67	35.55	0.26	30.30	7.23	1074.99	257.56
$S_1A_2T_5$	26.87	39.33	44.45	0.26	27.04	6.78	1203.30	300.97
$S_1A_2T_6$	31.60	45.34	33.33	0.21	25.31	6.39	816.15	211.73
$S_1A_2T_7$	33.27	47.66	31.11	0.15	23.01	5.75	715.64	178.88
$S_1A_3T_1$	36.80	44.67	37.38	0.14	24.67	7.16	909.52	281.37
$S_1A_3T_2$	35.47	41.00	44.45	0.21	24.08	7.45	1096.77	318.91
$S_1A_3T_3$	37.40	44.33	35.55	0.16	23.77	6.81	845.01	242.81
$S_1A_3T_4$	37.94	45.34	31.11	0.14	23.14	6.40	719.48	199.01
$S_1A_3T_5$	37.20	47.00	37.78	0.14	23.05	6.32	871.44	238.70
$S_1A_3T_6$	40.27	51.00	28.89	0.12	21.12	5.58	612.77	161.51
$S_1A_3T_7$	43.20	55.67	24.45	0.11	18.81	4.71	460.21	114.83
$S_2A_1T_1$	13.53	26.34	73.33	0.66	44.43	14.72	3199.11	740.22
$S_2A_1T_2$	13.00	23.00	82.22	0.74	43.58	14.25	3631.36	1172.35
$S_2A_1T_3$	14.67	24.33	73.33	0.60	42.85	13.00	3078.84	954.35
$S_2A_1T_4$	16.40	27.34	68.89	0.42	42.89	12.21	2906.15	842.05
$S_2A_1T_5$	16.07	28.66	75.56	0.42	38.92	10.18	3031.21	770.52
$S_2A_1T_6$	18.33	32.00	64.45	0.45	36.20	8.46	2333.45	545.04
$S_2A_1T_7$	19.93	35.33	57.78	0.33	34.06	7.63	1727.12	441.08
$S_2A_2T_1$	19.53	30.00	66.67	0.56	40.55	12.13	2525.93	807.47
$S_2A_2T_2$	18.53	28.33	75.55	0.60	37.85	11.87	3063.35	895.88
$S_2A_2T_3$	20.87	30.00	68.89	0.58	36.55	10.04	2521.19	690.45
$S_2A_2T_4$	21.27	30.33	73.33	0.50	35.10	9.16	2576.93	673.64
$S_2A_2T_5$	20.40	31.34	71.11	0.45	33.09	8.25	2352.69	587.25

Table 2: Continue...

Table 2: Continue...

$S_2A_2T_6$	22.47	35.00	55.55	0.41	30.13	7.90	1674.15	438.09
$S_2A_2T_7$	24.73	37.00	44.45	0.33	28.18	7.09	1249.24	315.19
$S_2A_3T_1$	28.33	34.00	57.78	0.42	34.96	10.13	2004.51	576.62
$S_2A_3T_2$	27.00	31.67	60.00	0.44	34.73	9.61	2098.58	585.76
$S_2A_3T_3$	29.13	33.33	48.89	0.42	31.67	9.24	1549.62	450.55
$S_2A_3T_4$	29.87	35.00	44.45	0.41	28.78	8.19	1280.20	364.70
$S_2A_3T_5$	32.00	35.00	48.89	0.41	26.65	7.34	1304.12	358.29
$S_2A_3T_6$	36.67	39.00	35.55	0.40	24.70	7.05	874.36	250.80
$S_2A_3T_7$	39.20	39.67	31.11	0.34	23.24	6.68	724.35	208.39
SE (m)	0.21	N.S	N.S	0.007	0.46	0.20	N.S	N.S
CD at 5 %	0.60	N.S	N.S	0.019	1.30	0.54	N.S	N.S

S_1: Flat; S_2: Stalk end up; A_1: Freshly extracted stone; A_2: 10 days after extraction; A_3: 20 days after extraction.

T_1: GA_3 - 100 ppm; T_2: GA_3 - 200 ppm; T_3: KNO_3 - 1 ppm; T_4: KNO_3 -2 ppm; T_5: Cow dung slurry; T_6: Water; T_7: Control (No treatment).

induces the denovo synthesis of proteolytic enzymes like ribonuclease and α-Amylase. The enzyme α-amylase and accompanying hydrolytic enzyme(s) successively hydrolyse the starch in endosperm thereby providing essential sugars for growth initiation processes and also liberate chemical energy which is utilized for RNA synthesis, activation of embryo as well as the suppression of inhibition which in turn resulted in higher germination (Copeland and Mcdonald, 1995). GA_3 treatment also have an ability to overrule the thermo-dormancy, photo dormancy, dormancy imposed by incomplete development of embryo, presence of various germination inhibitors as well as mechanical barriers in peach (Diaz and Martin, 1971).

In general, the regulation of growth by gibberellins and potassium nitrate relates virtually to its stem elongation properties, especially due to the enhancement of metabolites responsible for cell division and enlargement of cell. These chemicals act exclusively on stem elongation by loosening the cell wall, increasing the concentration of solutes by increasing the extensibility of cell wall, stimulating cell wall synthesis, reducing the cell wall rigidity and by increasing cell division leading to more efficient growth. The indirect effect caused by these chemicals on stem elongation is by increasing the IAA synthesis that leads to more vegetative growth (Leopold and Krieddemann, 1983).

Increase in dry weight of seedlings by GA_3 application might be due to the improved mobilization of the nutrients, which promotes the plant growth and development in better way. In khirnee the GA_3 treatment might have resulted into higher production of photosynthates and their translocation through phloem tissue to the root zone might have led to increase in the production of lateral roots thereby increasing the root length (Vachhani *et al*., 2014). The exogenous application of GA_3 also triggered the activity of gluconeogenic enzyme during the early stages of seed germination and this could be a probable reason for improved vigour characteristics which directly reflected on more production of lateral roots as well as increased root length, thereby improved the shoot growth in tamarind (Vasantha *et al.* 2014). This might have resulted in increased total dry weight of the seedling. In pea seedlings the cumulative effect of better root and shoot growth as well as more production of lateral roots have led to overall assimilation and redistribution of photosynthates within the plant system, thereby promoting the better growth and development (Brian and Hemming, 1955).

The better results with respect to vigour indices might be due to the cumulative effect of higher germination percentage, shoot length, root length and seedling dry weight under GA_3 treatment.

Interaction effect

Interaction effects (Table 2) also indicated that the sowing of freshly extracted stones by stalk end up method after treatment with 200 ppm GA_3 for 24 hours resulted in significantly higher germination rate (0.74) and minimum

Fig 3: Effect of pre sowing treatments on seedling length of freshly harvested mango stones sown in stalk end up method.

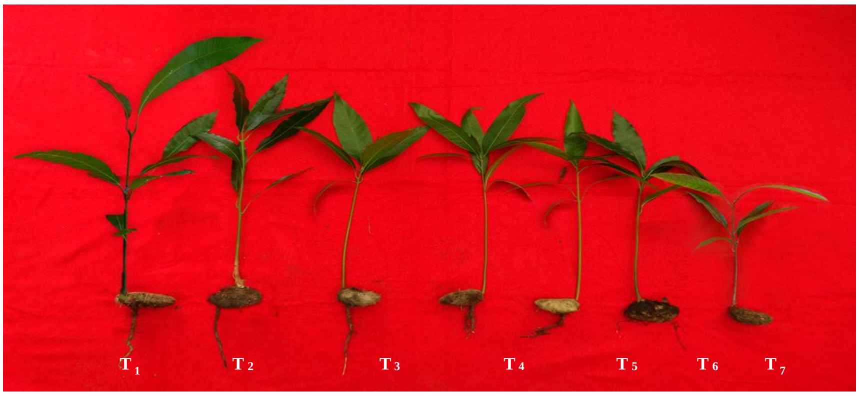

Fig 4: Effect of pre sowing treatments on seedling length of freshly harvested mango stones sown in flat method.

days for initiation of germination (13.00 days). Whereas the highest seedling length (44.43 cm) and dry weight (14.72 g) was recorded in stalk end up method after treatment with 100 ppm GA_3 for 24 hours (Fig 3). Sowing of stones 20 days after extraction from the fruit by flat method without any pre-sowing treatments resulted in poor germination and vigour of mango seedlings (Fig 4).

CONCLUSION

Germination became progressively slower as the age advanced. The micropyle positioning while sowing has direct implications on seedling quality as it determines the uniformity, speed as well as rate of germination. The GA_3 treatment resulted in earliest germination, boosting the growth, enhancing the seedling vigour and reducing the mortality. Hence the cumulative effect of viable and physiologically more active freshly harvested stones which were pre-treated with GA_3 and sown in micropyle pointing downward (stalk end up) position resulted in better germination and vigour of mango seedlings.

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