

Comparative Effect of Seed Priming and Growing Media on Germination and Seedling Rootstocks of Mango (*Mangifera indica*)

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Abstract

The poor seedling rootstock is a key factor in mango yield reduction. Container-based study was conducted to evaluate the effect of seed priming and growing media on seedling rootstocks of mango. Hydropriming of seed was performed by using distilled water, while gibberellic acid (GA3) and NPK fertilizer (Solo plant) were used for hormonal and nutripriming, respectively. In second part, the primed and unprimed seeds were planted in four different growing media (GM) including GM1, GM2, GM3 and GM4. Data showed that seed germination (%), germination index (GI), seedling vigor index (SVI), height of seedling, stem diameter, chlorophyll content, electrolyte leakage of leaf, nutrient (N, P, K, Ca and Mg) contents in leaf tissue was significantly altered by both seed priming and growing media respectively. In case of priming treatments, hormonal primed seeds had the best seed germination (77.01%), stem diameter (9.65 mm) and electrolyte leakage of leaf (13.01%); while N (1.13%), P (0.14%), K (0.87%), Ca (2.40%) and Mg (0.34%) content of leaf tissue was observed maximum in nutripriming treatment. Whereas, seed germination (71.96%), germination index (11.15) and stem diameter (9.41 mm) had greater values in response to the GM2 treatment. While GM4 grown seedlings had higher seedling height (33.81 cm), N (1.17%), P (0.15%), K (0.98%), Ca (2.64%) and Mg (0.38%) content of leaf tissue. It is concluded that seed germination and seedling growth attributes had a greater influence of GA3, while mineral nutrient contents of leaf had a significant effect on nutripriming. Among growing media, GM2 was observed better for growth parameters while GM4 for mineral nutrient contents of leaf..

Keywords: seedling rootstocks; hydropriming; nutripriming; growing media; germination index.

Introduction

Mango is generally propagated by sexual and asexual methods (Gholap and Polara, 2015; Pinto *et al.*, 2018). There are two distinct types of mango varieties including monoembryonic and polyembryonic. Seedling that is raised from monoembryonic seeds is not considered as true-to-type mango. Generally, both zygotic and nucellar seedlings may be used as a rootstock (Ballyise, 2006; Kolekar *et al.*, 2017). Typically, when mango plants are raised by seed, they lose their many unique features, consequently, vegetative propagation methods including grafting and budding become important to preserve and perpetuate the characteristics of each cultivar of mango (Abbas *et al.*, 2015). Healthy seedling rootstock is the main basis for successful and sustainable fruit production of mango in Pakistan (Kumar *et al.*, 2008). Seed priming technique and growing medium are considered as main starters for the seed germination and growth of the seedlings (Aklibasinda *et al.*, 2011; Aatlai and Srihari, 2013; Pinto *et al.*, 2008). Mango seeds have poor viability due its recalcitrant nature. According to (Gill *et al.*, 1985) the viability of the mango seeds is typically declined after

15-days of the fruit harvest. Mango seed initiates to germinate about twelve days after planting and may finish within thirty days of planting (Thakriya *et al.*, 2017). The delay in mango seed germination is typically due to its hard seed coat which is impermeable for water and gases and either due to deficiency or excess of growth hormones (Basra *et al.*, 2005). Seed priming is recognized as an effective and efficient method to improve seed germination potential and seedlings emergence (Shehzad *et al.*, 2012; Ramirez and Davenport, 2010). Usually, old mango seeds have low viability than fresh seeds. Storage of seeds may also reduce the germination potential (Khan *et al.*, 2006). Thus, seed priming can increase germination potential of mango seeds. Considering seed morphology and physiology, various seed priming methods have been formulated for improving its germination and emergence potential such as (a) hydropriming in which water used for soaking of seeds, (b) osmopriming where solutions having different osmotic are used for seed soaking, (c) halo priming in which seed is soaked with salt solution, (d) thermo-priming in which seeds of crop is treated under low or high temperatures extremes, (e) bio

priming, when seed is treated with inoculum and (f) nutripriming in which seed is treated in essential plant nutrients solution. Seed priming has been used commercially to enhance seed germination potential of several crops including field as well as fruit crops. However, this technique is less utilized to improve seed germination potential of mango crop. Another driving factor which plays key role in sustainable production of mango is use of growing media for seedlings establishment. The properties of growing media such as nutrients availability, water holding and cation exchange capacities are closely linked to its content of organic matter. Further, these media may also facilitate in root penetration and provide better aeration consequently improve mango rootstock seedlings (Supriya and Polara, 2015; Mhango et al., 2008). A wide variety of materials are being used and mixed in different ratios for formulation of growing media for the establishment of rootstock seedlings of mango. Those include organic

materials such as peat, sawdust, coconut husk, sugar mills industrial waste, etc. and inorganic materials such as sand, canal silt, perlite etc. (Donovan et al., 2016; Ryan et al., 2001). The composition and formulation of each growing medium may depend on type of crop, locality and its availability (Ryan et al., 2001). Many commercial growing media are available in the market of advanced countries for raising and establishing healthy seedlings of different crops. However, commercial media availability is a big hurdle for raising mango rootstock seedlings in under developed countries such as in Pakistan. Hence, this study was designed to formulate low-cost but having high nutrients availability growing media for mango rootstock seedlings.

Materials and Methods

Seed priming technique: The mango fruits of Sindhri cultivar were collected from the commercial orchards. The seeds were extracted and air-dried (Figure 1).



Figure 1. Drying of the mango seeds

The seeds were primed in various priming solutions and unprimed seeds were treated as control. The distilled water was used for hydropriming. Hormonal priming was performed by using gibberellic acid @ 100 mg L⁻¹ (Sigma Aldrich). The NPK fertilizer (solo plant) @ 100 mg L⁻¹ was used for nutripriming. The process of seed priming was conducted for 48 hours. The primed seeds were planted in polythene bags contained various growing media combination as described below.

Formulation of growing media: To prepare following growing media (GM) combinations, cheap materials available in the vicinity of Tandojam such as orchard soil, canal silt, bagasse and press mud from sugar mill industry were collected from local sugar mill to

formulate following GMs (Figure 2 & 3). GM₁: Orchard soil (farmer's practice)

GM₂: 70% Bagasse + 5% Coco peat + 25% Canal silt

GM₃: 60% Bagasse + 5% Coco peat + 35% Canal silt*

GM₄: 20% Bagasse + 20% Coco peat + 30% Canal silt + 30% Press mud

*GM₃–Standard practice developed under ASLP (Australian Sector Linkage Program) project at Mango Research Station Shujabad.

Soil analysis: The composited soil and canal silt samples were collected brought in the laboratory, air-dried and ground by using 2 mm sieve. The electrical conductivity (EC) and pH of the soil-water extract (1:5) were measured with pH and EC meters respectively. Walkley-Black method was used for the measurement of organic

matter (OM) content. The total Nitrogen using Kjeldahl's distillation, whereas Olsen method was used for determination of available Phosphorus. Flame

photometric technique was used for the measurement of exchangeable K, Ca and Mg (Bremner, 1965).



Figurer 2. Substrates for the growing media



Figure 3. Mixing of the media substrates

Analysis for growth media components: The pH and EC of the bagasse, cocopeat and press mud were measured using saturated media extract (SME) method followed by pH and EC meters were used. Total Nitrogen of bagasse, cocopeat and press mud was analyzed by Kjeldahl's method (Richards, 1954). The P (Estefan *et al.*, 2013), K, Ca and Mg contents were measured by using atomic absorption spectrophotometer.
Leaf tissue analysis: Fully matured leaves were collected from grown seedlings. The leaves were brought to laboratory, washed; oven dried (68°C) and wet-digested in mixture of perchloric (HClO₄) and nitric acid (HNO₃) at the ratio of 1:5 and filtered. The filtrate was used for analysis of Phosphorus by using

vanadomolybdo-phosphoric acid method (Cottenie, 1980) whereas flame photometer was used for analysis of potash (Knudsen *et al.*, 1982). The EDTA method was used for the determination of Ca and Mg (Knudsen *et al.*, 1982).

Plant data: The plant data was recorded for seed germination (%) (GP) mean germination time (MGT), germination index (GI), seedling vigor index (SVI), height of seedlings, stem diameter and chlorophyll contents. Seed germination was recorded for one month at interval of one week. The seed germination (%) was computed by following formula (Larsen and Andreasen, 2004).

$GP = \frac{\sum n}{N} \times 100$ (where n: No. of germinated seeds, N: total No of planted seeds).

The MGT was determined by following formula:

$$MGT = \frac{\sum Dn}{\sum n}$$

(where n: No. of germinated seeds on day D and Dn: No. of days counted from the initiation of seed germination)

The GI was computed using formula (Association of Official Seed Analysts. 1983).

$$GI = \frac{MGT = \frac{\sum Dn}{\sum n}}{\frac{\text{Number of germinated seeds}}{\text{Days of first count}} + \frac{\text{Number of germinated seeds}}{\text{Days of last count}}}$$

The five random seedlings of each treatment were selected for seedlings height. The seedlings height was measured from tip to the base of the plant at 30 days interval. Stem diameter (mm) was determined by using a digital vernier caliper at three points (center, top, and bottom of the stem). Chlorophyll content was determined by using SPAD 502.

Statistical analysis: The entire data so collected were statistically analyzed by using Statistix Software package (Ver. 8.1). The effects of the main factors and their interaction with each other were determined. Experiment was laid out according in completely randomized design factorial. The superiority of the treatments was evaluated by applying Least Significant Difference test at $p < 0.05$.

Results

The means of the seed priming depicted greater variation in results of the seed germination percentage, Germination index, and Seed vigor index (Table 1). Each seed priming treatment had a germination percentage of more than 50% except nutriprimed seeds which had germination of 48.61%, even lower than control (68.81%). The greatest mean for seed germination (77.01%), germination index (10.82) and seedling vigor index (4271.75) was observed in hormonal primed seeds. However, germination index was also similar to the

results obtained from hydro (10.71) and nutriprimed (11.08) seeds. The SVI was also found greater in unprimed seeds (2490.60) as compared to nutriprimed seeds (2151.37). In case of growing media effect (Table 1), seed germination was observed more than 50% from each growing media producing the greatest (71.96%) in GM₂. However, the germination index (11.15; 10.95) and SVI (3147.87; 3170.72) was observed similar to GM₂ and GM₄, respectively. The minimum seed germination (57.84 %), germination index (8.80) and SVI (2550.90) noticed in control treatment. In case of interaction between seed priming and growing media, only SVI produced significantly the greatest seedling vigor index (4875.1) in hormonal seed priming and GM₂. Further, similar response (4436.4) was also observed in hormonal seed priming and GM₄ treatment.

The seedling height and stem diameter were significantly altered by seed priming and growing media treatments (Table 2). However, interaction of both seed priming and growing media treatments had no effect on seedling height and stem diameter of mango seedlings. The mean seedling height ranged from 23.15 (control) to 35.45 cm (hormonal priming). Hormonal seed priming also produced greater stem diameter (9.65 mm) than any other primed treatment. Moreover, seedling height and stem diameter in hormonal priming was also found significantly different from nutripriming (33.28 cm; 8.59 mm) and hydropriming (29.30 cm; 8.16 mm) treatments. In case of growing media (Table 2), seedlings height followed an order of GM₄ > GM₃ > GM₂ > GM₁. Whereas, stem diameter of rootstock seedlings planted in different growing media followed a different growth trend. Stem diameter of seedlings ranged from 7.72 mm (GM₄) to 9.41 mm (GM₂).

Table 1. Seed priming and growing media effects on mango seed germination and seedling vigor index.

Seed priming	Growing media (GM)				Mean
	GM ₁	GM ₂	GM ₃	GM ₄	
Seed germination (%)					
Unprimed seeds	61.19	79.28	62.90	71.85	68.81B
Hydro priming	54.60	69.34	60.61	57.88	60.61C
Hormonal priming	71.78	83.61	73.11	79.56	77.01A
Nutripriming	43.79	55.62	48.61	46.42	48.61D
Mean	57.84C	71.96A	61.31BC	63.93B	
Germination Index (GI)					
Unprimed seeds	7.41	8.64	8.70	7.84	8.15B
Hydro-priming	9.67	11.34	10.83	10.99	10.71A
Hormonal priming	9.14	11.96	9.57	12.60	10.82A
Nutripriming	8.98	12.68	10.27	12.37	11.08A
Mean	8.80C	11.15A	9.84B	10.95A	
Seedling Vigor Index (SVI)					
Unprimed seeds	1970.8fg	2903.9de	2156.1fg	2931.6de	2490.60B
Hydro-priming	2426.5def	2958.6d	2041.8fg	2908.7de	2583.90B
Hormonal priming	3856.9c	4875.1a	3918.6bc	4436.4ab	4271.75A
Nutripriming	1949.4fg	1853.9g	2396.0efg	2406.2ef	2151.37C
Mean	2550.90B	3147.87 A	2628.12B	3170.72A	

Table 2. Seed priming and growing media effects on seedling height and stem diameter of mango seedlings.

Seed priming	Growing media (GM)				Mean
	GM ₁	GM ₂	GM ₃	GM ₄	
Seedling height (cm)					
Unprimed seeds	20.67	22.12	23.98	25.84	23.15D
Hydro priming	26.16	27.99	30.35	32.70	29.30C
Hormonal priming	31.66	33.87	36.72	39.57	35.45A
Nutripriming	29.72	31.80	34.47	37.15	33.28B
Mean	27.05D	28.94C	31.38B	33.81A	
Stem diameter (mm)					
Unprimed seeds	5.91	8.12	6.32	6.61	6.74C
Hydro priming	7.48	9.35	8.00	7.82	8.16B
Hormonal priming	9.05	11.04	9.68	8.82	9.65A
Nutripriming	8.49	9.13	9.09	7.64	8.59B
Mean	7.73B	9.41A	8.27B	7.72B	

The mean chlorophyll contents and electrolyte leakage significantly differed in seed priming treatments (Table 3). The interaction of seed priming and growing media effect was only significant on leakage of the electrolytes from leaf. The hormonal primed seeds produced seedlings with higher mean chlorophyll content (53.76rg) followed by nutripriming (51.14 rg). Unprimed seeds produced seedlings with lower chlorophyll content (35.64 rg) and with more leakage of the electrolytes (16.10%). The effect of growing media on chlorophyll content indicated that GM₂(47.88 rg) and GM₃(49.31 rg) grown seedlings were statistically similar than GM₁ (42.51 rg) and GM₄ (45.50 rg). The interaction effect of seed priming and growing media revealed that unprimed

seedlings had greater leakage of the electrolytes (17.23%) than GM₃. The N content of leaf was significantly differed by both seed priming and growing media (Table 4). The interaction of seed priming and growing media found non-significant. The means of the seed priming depicted that N content of leaf was more than 1% from each priming treatment with maximum (1.13%) from the seedlings grown in nutripriming. The seedlings from unprimed (1.03%) and hydroprimed (1.04%) seeds had a similar N content of the leaf. On the basis of growing media, the mean N content of GM₁ was less than 1% i.e. 0.92%. The GM₄ grown seedlings had more N content (1.17%) following by GM₂ (1.08%).

Table 3. Seed priming and growing media effects on Chlorophyll content and leakage of the electrolytes in mango seedlings.

Seed priming	Growing media (GM)				Mean
	GM ₁	GM ₂	GM ₃	GM ₄	
Chlorophyll content (rg)					
Unprimed seeds	32.48	36.59	37.68	35.80	35.64D
Hydro priming	41.11	46.31	47.69	43.50	44.65C
Hormonal priming	49.74	56.03	57.70	51.59	53.76A
Nutripriming	46.70	52.60	54.17	51.12	51.14B
Mean	42.51C	47.88AB	49.31A	45.50B	
Electrolyte leakage (%)					
Unprimed seeds	16.16ab	14.26abcd	17.23a	16.75a	16.10A
Hydro priming	16.87a	12.65cd	11.40d	11.52cd	13.11B
Hormonal priming	11.41d	14.69abc	13.23bcd	12.70cd	13.01B
Nutripriming	14.52abcd	14.40abcd	12.98bcd	11.97cd	13.47B
Mean	14.74	14.00	13.71	13.23	

Table 4. Seed priming and growing media effects on leaf N content (%) in mango seedlings.

Seed priming	Growing media (GM)				Mean
	GM ₁	GM ₂	GM ₃	GM ₄	
Unprimed seeds	0.89	1.05	1.03	1.14	1.03C
Hydro priming	0.90	1.06	1.04	1.15	1.04BC
Hormonal priming	0.90	1.07	1.05	1.16	1.05B
Nutripriming	0.98	1.16	1.14	1.26	1.13A
Mean	0.92D	1.08B	1.06C	1.17A	

The P and K contents of leaf were significantly differed by both seed priming, growing media and their

interaction (Table 5). Hydro and hormonal primed grown seedlings had similar mean results for P (0.13; 0.13%)

and K (0.79; 0.80%). While unprimed grown seedlings had less P (0.12%) and K (0.71%). The maximum P (0.14%) and K (0.87%) content of leaf were observed in nutripriming. The GM₄ grown seedlings had maximum

leaf P (0.15%) and K (0.98%). The GM₂ and GM₃ had similar leaf content of P (0.13; 0.13%) and K (0.90; 0.89%).

Table 5. Seed priming and growing media effects on leaf P and K contents (%) in mango seedlings.

Seed priming	Growing media (GM)				Mean
	GM ₁	GM ₂	GM ₃	GM ₄	
Phosphorus content of leaf (%)					
Unprimed seeds	0.07g	0.13de	0.13de	0.14bc	0.12C
Hydro priming	0.11f	0.13de	0.13de	0.14b	0.13B
Hormonal priming	0.11f	0.13cd	0.13de	0.14b	0.13B
Nutripriming	0.12e	0.14b	0.14bc	0.16a	0.14A
Mean	0.10C	0.13B	0.13B	0.15A	
Potassium content of leaf (%)					
Unprimed seeds	0.14g	0.88cde	0.86e	0.95bcd	0.71C
Hydro priming	0.47f	0.88cde	0.87e	0.95bc	0.79B
Hormonal priming	0.48f	0.89bcde	0.87de	0.96b	0.80B
Nutripriming	0.52f	0.97b	0.95bcd	1.05a	0.87A
Mean	0.40C	0.90B	0.89B	0.98A	

The Ca and Mg content of leaf were also significantly affected by seed priming and growing media and their interaction (Table 6). The interactive effect of seed priming with each growing media depicted that seedlings had Ca content of more than 2% in each treatment of the interaction except the interaction of GM₁ and unprimed seeds. The Ca content of GM₁ grown seedlings range from 1.20 to 1.60% with a mean value of 1.44%.

However, seedlings raised in GM₂, GM₃ and GM₄ media depicted similar results of Ca and Mg in response to the unprimed or hydro or hormonal primed seeds. Only seedlings grown from nutriprimed seeds produced better results in each growing medium. Seedlings had maximum mean Ca (2.40%) and Mg (0.34%) content in nutripriming; while GM₄ had better mean results for Ca (2.64%) and Mg (0.38%).

Table 6. Seed priming and growing media effects on leaf Ca and Mg contents (%) in mango seedlings.

Seed priming	Growing media (GM)				Mean
	GM ₁	GM ₂	GM ₃	GM ₄	
Ca content of leaf (%)					
Unprimed seeds	1.20f	2.36c	2.32c	2.56b	2.11C
Hydro priming	1.46e	2.38c	2.34c	2.58b	2.19B
Hormonal priming	1.48e	2.40c	2.36c	2.60b	2.21B
Nutripriming	1.60d	2.61b	2.56b	2.83a	2.40A
Mean	1.44C	2.44B	2.40B	2.64A	
Mg content of leaf (%)					
Unprimed seeds	0.15f	0.34c	0.33c	0.37b	0.30C
Hydro priming	0.21e	0.34c	0.33c	0.37b	0.31B
Hormonal priming	0.21e	0.34c	0.34c	0.37b	0.31B
Nutripriming	0.23d	0.37b	0.36b	0.40a	0.34A
Mean	0.20C	0.35B	0.34B	0.38A	

Table 7. Critical level/range of nutrient contents in mango leaves reported by the different scientists.

Nutrient element	Samra et al. 1978	Young & Koo (1971) Young & Sauls (1981)	Catchpole & Bally (1995)	Robinson et al. (1997)	Poffley & Owens, 2005
N (%)	0.95-1.45	1.00-1.50	0.80-1.90	1.00-1.50	0.8-1.2
P (%)	0.03-0.12	0.09-0.18	0.12-1.30	0.080-0.18	0.08-0.18
K (%)	0.40-0.77	0.50-1.00	0.40-2.50	0.30-1.20	0.4-1.2
Ca (%)	1.74-3.45	3.00-5.00	1.50-2.80	2.00-3.50	1.5-2.8
Mg (%)	0.22-0.75	0.15-0.47	0.20-0.40	0.15-0.40	0.2-0.4

Discussion

Seed priming is a widely accepted method to achieve uniform germination and high-quality seedlings of crops (Ma *et al.*, 2018). In view, different seed priming techniques were compared to observe the response of mango rootstock seedlings to hormonal, nutripriming and hydropriming techniques. The results of present study indicated that hormonal priming showed promising results for enhancing seed germination percentage, germination index, seedling vigor index, seedling height, stem diameter, chlorophyll content and electrolyte leakage of the leaf than rest of the priming techniques. This might be due to gibberellic acid (GA₃) role in the stimulation of amylase synthesis and production that could hydrolase starch into endosperm and provide sugars, consequently encouraged seed germination (Matilla *et al.*, 2008; Voegel *et al.*, 2011). According to Kolekar *et al.*, (2017) the application of GA₃ (@100 ppm) could increase seed germination and SVI of mango. Similarly, Venkat and Reddy (2005) and Shaban (2010) indicated that 100 or 200 ppm amount of GA₃ could be beneficial for improving seed germination of rootstock seedlings of mango. In some other studies, it has been indicated that greater rates (i.e. 500 to 1000 ppm) of GA₃ may be considered to improve seed germination of rootstock seedlings of mango (Abbasi *et al.*, 2019). Further, GA₃ also increased plant height, stem girth and number of leaves of mango as in this study. This was likely due to alteration in meristematic tissues of mango (Venkat *et al.*, 2006; El-Zaher, 2008). Further, response of GA₃ may also be differed in varieties of mango due to differentiation in development of meristematic tissues of mango (Mobli and Baninasab, 2008). The increase in chlorophyll content of leaves in GA₃ primed rootstock seedlings as compared to other seed priming techniques in present study was attributed due to increased rate of photosynthesis. Further, the increased chlorophyll content was likely due to involvement of growth hormones in the synthesis of chlorophyll molecule (Kanjilal *et al.*, 1998; Shah, 2007). Some studies also showed that the synthesis of chlorophyll content of rootstock seedlings of mango was also affected by the rate of GA₃ (Mostafa and Alhamd, 2011; Jayantilal, 2015).

The production of rootstock of superior quality is the pre-requisite for plantation of a mango orchard (Mngomba *et al.*, 2010; Kaur, 2017). In this study, various growing media were compared to formulate low cost and good quality growing media for rootstock seedlings of mango. Among all tested growing media, GM₂ produced better seed germination (%), GI, SVI, stem diameter and chlorophyll content. These results were likely due to increased aeration and drainage properties by coco peat and bagasse materials (Sarkar *et al.*, 2005). Generally, bagasse and coco peat may improve physical and chemical properties of growing

media and resultantly improved seedlings development in nursery (Abad *et al.*, 2002; Basirat, 2011). In recent study (UIHaq *et al.*, 2017) indicated greater mango rootstock seedling survival in growing medium having bagasse (70%) as major ingredient. In contrast, Memon *et al.* (2017) observed non-significant differences in growth parameters of mango rootstock in media containing low amount of bagasse in mixture.

The nutrient content is also one of the key criteria for selection of growing media for nursery development. In present study, leaf N content in seedling of mango rootstock was found in the range of the critical levels mentioned in Table 7 (Young and Koo, 1971; Samra *et al.*, 1978; Catchpole and Bally, 1995; Robinson *et al.*, 1997; Poffley and Owens, 2005). Further, P and K levels were also found in the established ranges of mango seedlings. However, Ca content was noticed low as compared to previously reported levels (Young and Koo 1971; Young, and Sauls, 1981) while level of Mg found satisfactory. Further, these typical differences in critical levels of nutrients might be linked to combination of rootstock and scion, time of sampling, age of plant, propagation mode and varieties of mango (Ryan *et al.*, 2001; Zuazo *et al.*, 2006).

Conclusion

It was concluded that seed germination (%) and seedling growth attributes of mango had a greater influence of gibberellic acid seed priming, while mineral nutrients content of leaf was significantly affected by nutripriming than any other seed priming technique. Among growing media, GM₂ was observed better for growth parameters while GM₄ for mineral nutrients content of leaf.

Competing Interests Disclaimer

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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