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COMPARATIVE STUDY OF ROOTSTOCKS AND THEIR EFFECTS ON STIONIC ESTABLISHMENT OF THE MANGO SEEDLINGS

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ABSTRACT

To explore the quality rootstock which impart better stionic establishment of the mango seedlings, we studied the comparative effect of scion and rootstock using three mango scion varieties (Sindhri, Chaunsa and Neelum) grafted on three rootstocks i.e. Ratam, Pado and Sindhri. Parameters including seed germination (%), seedling height (cm), leaves seedling rootstock^{-1}, seedling vigor index (SVI), fresh biomass of shoot and roots (g), dry biomass of shoot and roots (g), chlorophyll content (rg), rootstock diameter (mm), scion diameter (mm), stionic ratio, leaves graft^{-1} and sturdiness quotient were studied. Results showed the best seed germination (88.33%), seedling vigor index (5158.74), and sturdiness quotient (3.89) in Pado rootstock. However maximum fresh biomass of shoot (20.67 g), fresh biomass of roots (16.2 g), dry biomass of shoot (12.65 g), dry biomass of roots (8.33 g) and scion diameter of the rootstocks were observed in Sindhri rootstocks. Regarding grafting data, the best stionic ratio (0.93) was observed from the scion and rootstock combination of Neelum and Pado rootstock. It was concluded that Pado rootstock performed better in terms of seedling rootstock and for successful stionic establishment of the mango seedlings.

Key words: *Rootstock; biomass; chlorophyll contents; Sindhri; sturdiness quotient; Mangifera indica*

INTRODUCTION

Mango (*Mangifera indica* L.) belongs to the Anacardiaceae family and was first grown in India about 5000 years ago. Their seeds traveled from Asia to Middle east, East Africa and south America. It is known as the king of fruits because it is nutritionally very rich, unique in taste and aroma, making up approximately half of all tropical fruits produced worldwide (Chauhadry *et al*., 2016). Due to its excellent taste, delicious flavor and high nutritional value, this crop is highly valued for both food and nutrition security, especially for developing countries where achieving food and nutritional security remains a challenge (Ullah *et al*., 2010). Mango is a highly cross-pollinated heterozygous plant. In ancient times, mangoes were propagated mainly by stones. Stone-propagated plants have a long juvenile phase and poor performance (Bobade *et al*., 2018). Therefore, it must be propagated vegetatively to produce true-to-type off-spring. In addition, most improved mango varieties are mono-embryonic, so grafting is required to produce a true type tree. Commercial mango propagation is done by grafting. This involves joining two genetically distinct parts of the plant with a grafting compound to form a complete tree. These two parts are called scion (fruit system) and rootstock (root system). These rootstocks are usually established by growing seeds of non-grafted (desi) plants. In recent years, Pakistani mango plantations have experienced certain physiological stresses and quality problems: unbalanced fertilization, micronutrient deficiencies, poor tree management and inappropriate planting methods of rootstock. Inadequate selection of orchards has been observed as a major cause of orchard-related quality problems (Cicero *et al*., 2015). Among all these encountering factors, healthy rootstocks and their effects on stionic establishment are an essential start for establishing new mango orchards. The production of healthy rootstocks and their effects on grafting is the main key for successful orchard establishment. The seedling rootstock contributes prominently in success of grafts it serves as the bases that attached with soil and provide support, food and water to grafts (Pereira *et al*., 2004). They are a major food source and provide the root system of grafted plants. In general, rootstocks can be distinguished into two categories: seedlings and clone rootstocks. Plants propagated vegetatively by grafting and budding are considered clonal rootstocks, whereas seedling rootstocks are obtained by seed and may represent plant genetic variation (Minja *et al*., 2017). The success of grafting is primarily based on the use of the correct selection

of rootstock and growth. When the rootstock and scion are united to continue their growth as one plant, then the graft is also termed as stion. The stionic establishment of the seedlings is greatly affected by the multiple factors mainly includes selection of the vigorous rootstock and their compatibility with the scion cultivars. Correct alignment of the ridge and root tissues also determines the success of the graft (Akinnifesi *et al*., 2015; Ruiz *et al*., 2015). A strong and compatible rootstock plays a major role in modern mango production as it influences the tolerance to adverse abiotic and biotic soil stresses and the general horticultural characteristics of the grafted scion. Stionic relationship is major factor for the success of grafting. If the scion is from the dwarf plant and the rootstock is from a tall plant or vice versa, the graft union may occur but the growth of both the scion and stock may not be uniform. The non-uniform growth may affect the growth and development of the future plants, flowering, fruiting and ultimately on the total production. Selection of an appropriate rootstock and better scion and rootstock combination is crucial for the successful orchard establishment of mango. There is a lot of scope to increase the mango fruit production in the country by improving newly introduced commercial varieties through the adoption of modern vegetative propagation method with proper stionic relationship. But the information or research work regarding this in the country is very much lacking. Therefore, it is the time to look for appropriate stionic relationship and to obtain true-to-type planting materials, which is pre-requisite for strengthening the mango industry.

MATERIALS AND METHODS

A container-based experiment was conducted in the shade house during 2021-22 at Horticulture Garden, Sindh Agriculture University, Tandojam to compare different rootstocks and their effects on the stionic establishment of the mango seedlings. Two factors viz. rootstocks and scion varieties were compared under the present study to evaluate the efficacy of each factor independently as well as on the interaction basis. In factor-A rootstocks, stones of two non-grafted (Ratam and Pado) and grafted (Sindhri) were sown to establish seedling rootstocks for the grafting. The quality seedlings related attributes were monitored regularly. In factor-B scion varieties viz. Sindhri, Chaunsa and Neelum were obtained and evaluated for the best stionic establishment and survival of the seedlings. The experiment was laid out factorial in completely Randomized Design (CRD) with three replications. The experiment was conducted under the agro-ecological conditions of Tandojam at the Horticulture Garden, Sindh Agriculture University Tandojam, Sindh Pakistan. The site is located at 25º25’ 60’N 68º31’ 60’E 19.5 m elevation.

Raising of rootstock seedlings: Mango fruits were harvested from healthy commercial mango orchards in May-June. Seeds were collected from well ripened fruits and dried under shade at room temperature for one week. Dried mango seeds were washed with the fungicide solution (Topsin-M) for three minutes and the soaked stones were immediately planted in the growing media. The growing media was prepared by mixing decomposed bagasse, coco peat and canal silt at 65 + 5 + 30% respectively. The solarization of each growing medium @ 55-60 ^{0}C was done for one to two hours by using clear plastic sheet. The plastic bags of size 9 x 13 inches were used by maintaining drainage holes at the bottom side of the bag. The bags were filled with the growing media leaving one inch at the top of the bag to settle down the water level. Mango seeds were planted in the shade house to protect plants from insect pest and frost. To avoid disease contamination through contact with soil, a barrier layer of 15-25 cm is developed by using gravels over the soil surface.

Grafting operation*:* The raised rootstocks from various seed sources having good stem diameter were used as rootstock for the grafting. However, scions of Neelum, Sindhri and chaunsa varieties were taken from healthy, disease free and non-flowering shoots of the trees. The scion diameter of each variety was noted (Sindhri -6.28 to 8.29mm, Chaunsa -7.11 to 10.26 mm and Neelum – 6.67 to 7.28 mm). Each scion variety was grafted by using veneer grafting method.

Observations recorded: The following observations wwere recorded for seed germination (%), seedling height (cm), leaves seedling rootstock^{-1}, seedling vigor index (SVI), fresh biomass of shoot and roots (g), dry biomass of shoot and roots (g), chlorophyll content (rg), rootstock diameter (mm), scion diameter (mm), stionic ratio, leaves graft^{-1} and sturdiness quotient

Germination of the seeds was recorded weekly for upto one month. The germinated seeds were counted and percentage was calculated by applying following formula (Larsen and Andreasen, 2004).

$$\mathbf{GP = \sum n/ N \times 100}$$

The leaves per seedling rootstock were counted at 15 days interval of 50% random samples and continued up to six months. The mean values were calculated. The height of the seedling rootstock was measured from 50% random taken plants and means were calculated and expressed in centimeters. The height was measured with scale from soil surface or base of the plant to the growing tip of the plant.

The seedling vigor index was calculated from six months old seedlings of 50% random samples by using the following formula, Abdul-Baki and Anderson (1970)**.**

Vigor index (VI) = [seedling length (cm) × germination percentage]

The chlorophyll content of rootstock seedlings leaves was determined from six months old seedling rootstocks of 50% random samples from each treatment. The same was also determined after two months of grafting. It was determined with a portable chlorophyll meter using SPAD 502 and the values were expressed as relative greenness (rg).

Sturdiness quotient reflects the stocky or bushy nature of seedlings Thompson *et al*., (1985). Strength determined by dividing seedling height (cm) by neck diameter (mm) according to Roller (1977).

$$SQ = \frac{\text{Seedling height (cm)}}{\text{Collar diameter (mm)}}$$

The diameter of the seedling rootstocks was measured by digital vernier caliper at middle part of the rootstock of 50% random samples and values expressed as millimeters.

The fresh biomass of shoot and roots of 50% random samples were taken from each treatment and measured by using an electronic weighing balance and expressed in grams. Fresh shoots and roots were placed in pyrex beakers and dried at 70°C in an oven for 48 h until constant weight was reached. The dry weight of shoots and roots was then measured using an electronic balance and the values were expressed in grams. Stionic relationship is most crucial attribute to evaluate success of graft. The ratio was measured by dividing the number of sprouted grafts with the total number of grafted plants after bud burst. The leaves per graft were counted at 15 days interval and continued up to two months. The mean values were calculated. The diameter of the various scions was measured at 4-6 cm above the grafting union of 50% random samples. The diameter was measured by using digital vernier caliper and values expressed as millimeters. The chlorophyll content of the grafted seedlings was determined from two months old seedlings of 50% random samples from each treatment. It was determined with a portable chlorophyll meter using SPAD 502 and the values were expressed as relative greenness (rg).

Statistical analysis*:* Computer software (**Statistix, 2006**) was used for primary data analysis whereas. The LSD test was applied to compare treatment superiority if results were significant at the $P \leq 0.05$ probability level.

RESULTS

Results showed that Pado seed (desi) had more than 88% seed germination followed by Ratam (65%) and Sindhri (61%). Both Ratam (non-grafted) and Sindhri (grafted) had statistically similar results for seed germination (Figure 1a). Similar results were reported by **Minja *et al*., (2017)**. They observed different seed germination from various rootstocks maximum seed germination recorded in Ngwangwa (92.7%) followed by Zizi (69%) and lowest in Sindano (17.4%). Prajapati *et al*., (2012) observed the highest germination (57.18%) in local variety as compared to the Badam variety (28.41%). This difference in the results may be due to the various rootstocks.

The seedling height and number of leaves exhibit similar response to the seed source varieties. Each seedling source produced seedlings with similar height and similar number of leaves that ranges from 54.69 to 58.42 cm (Figure 1b) and 19 to 20.67 leaves (Figure 1c) respectively.

The seedling vigor index is the stability of the seedlings. It had significant differences in response to seed source varieties whether grafted or non-grafted (Figure 1d). The Pado seedlings had maximum seedling vigor index (5158.74) followed by 3529.33 from Ratam. The lowest seedling vigor index 3398.52 was observed from Sindhri.

Results have depicted that seedling produced from different seed sources had significant effects on the fresh and dry biomass of shoot and root. Maximum and similar fresh biomass of shoot (19.67; 20.67 g) were recorded from grafted Sindhri and non-grafted Pado varieties. Whereas the lowest fresh biomass of shoot (15.03 g) was recorded from non-grafted Ratam variety (Figure 2a). The fresh biomass of roots ranges from 6.27 g to 16.2 g. Sindhri variety produced seedlings with maximum fresh biomass of roots (16.2 g) followed by Pado (8.02 g) and Ratam (6.27 g) (Figure 2b). Figure 2c showed that seeds of Sindhri variety produced seedlings with maximum dry biomass of shoot (12.65 g) followed by Pado (8.96 g) and Ratam (6.19 g). Non-grafted and grafted seed sources had also highly significant differences for dry biomass of the roots that ranges from 2.01 to 8.33 g. Sindhri variety produced seedlings with maximum dry biomass of roots (8.33 g) followed by Pado (2.95 g) and Ratam (2.01 g). The mean sample of Sindhri variety had maximum standard error as compared to Pado and Ratam (Figure 2d).

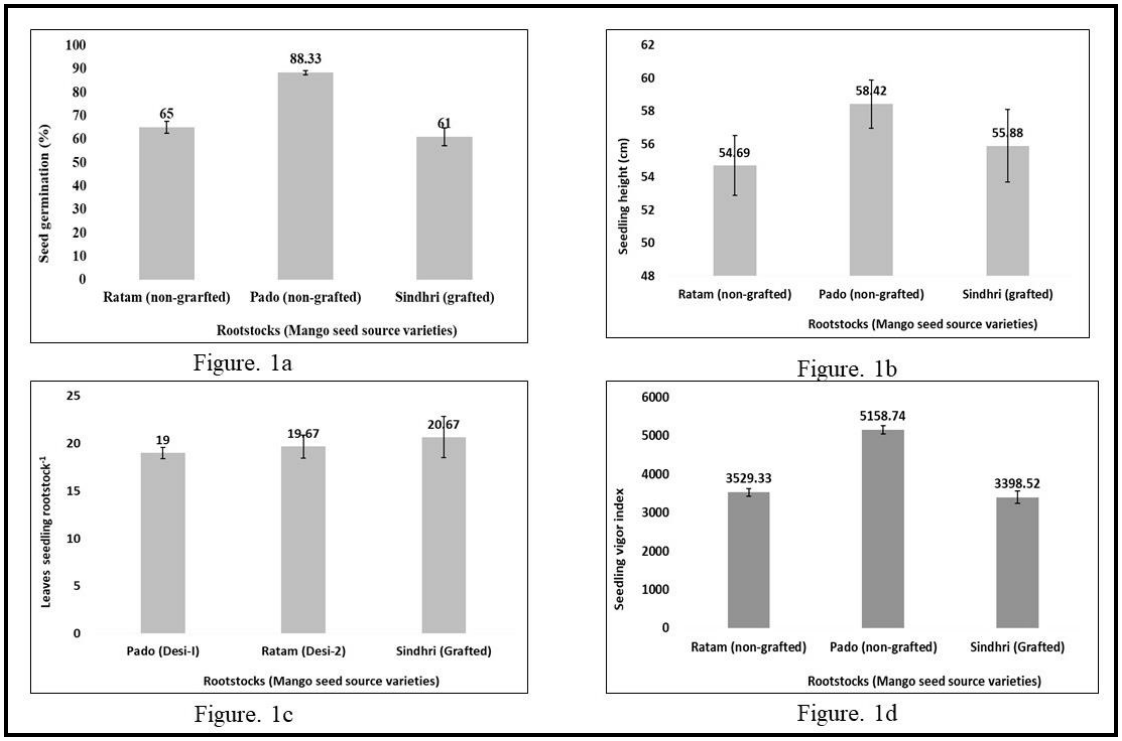

Figure 1. Performance of various growth parameters of mango varieties. Different letters indicate significant differences at $p \leq 0.05$. Vertical bars represent ± SE of means

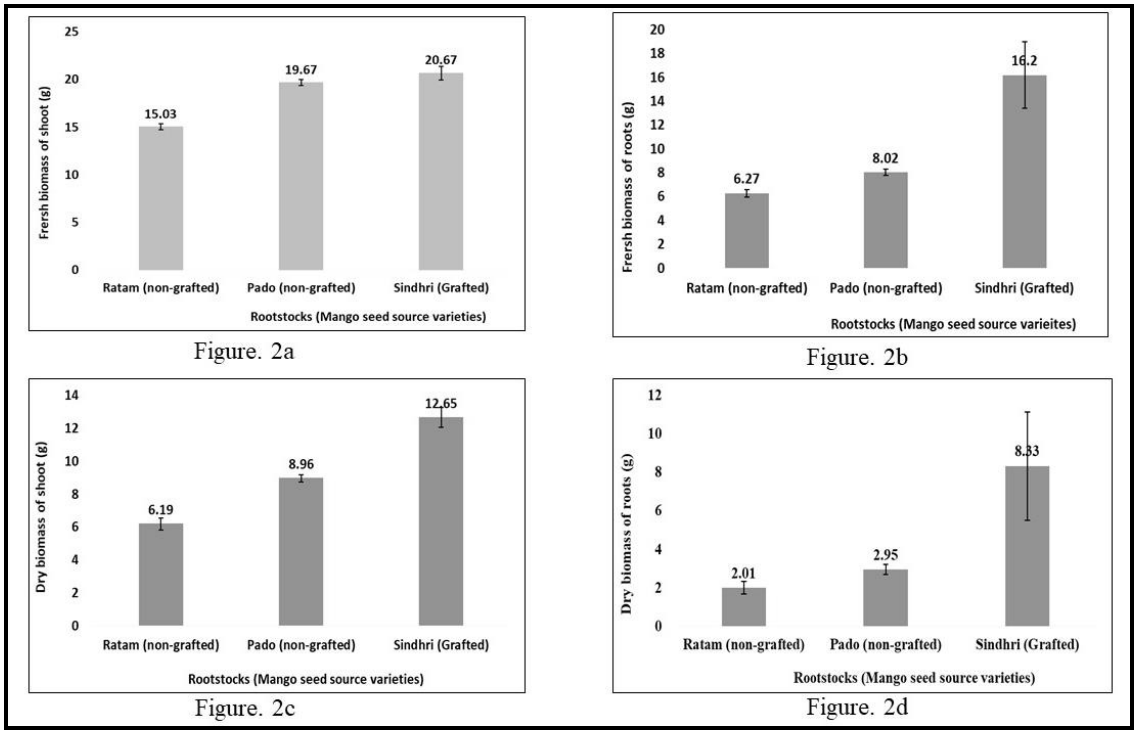

Figure 2. Performance of various fresh and dry biomass of shoot and root of mango varieties. Different letters indicate significant differences at $p \leq 0.05$. Vertical bars represent ± SE of means.

The chlorophyll content of the rootstock and grafted seedlings have statistically similar response to various seed source (rootstock). However, chlorophyll content of grafted seedlings wassignificantly affected by scion varieties. The chlorophyll content of the rootstock seedlings ranges from 31 to 33.63 rg (Figure 3a). To compare means of the scion varieties, Sindhri (23.42 rg) and Neelum (22.42 rg) varieties had similar chlorophyll content of the leaves. The scion of Chaunsa variety had the lowest chlorophyll content (14.18 rg) of the leaves (Figure 3b). The chlorophyll content was also significantly affected by the rootstock and scion combinations.

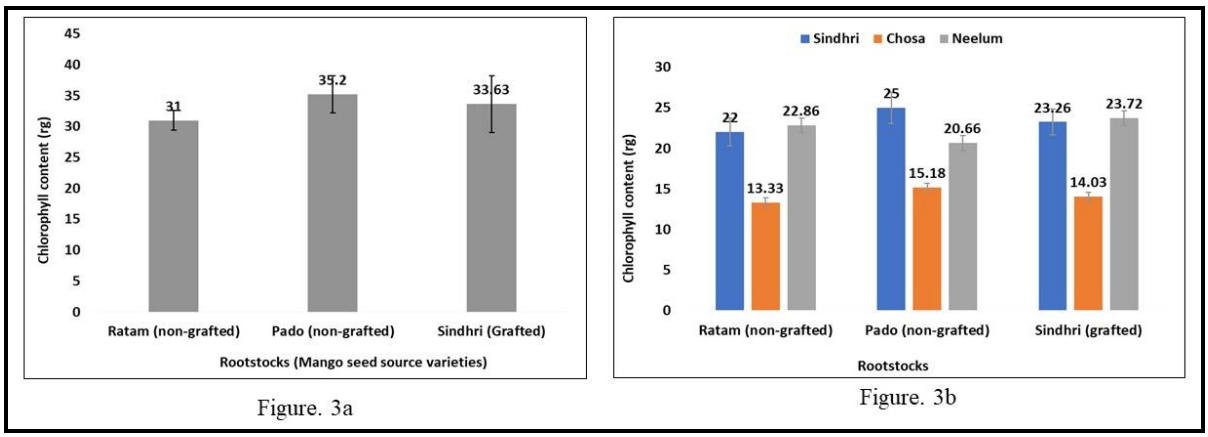

Figure 3. Performance of chlorophyll content of various rootstock and scion varieties of mango. Different letters indicate significant differences at $p \leq 0.05$. Vertical bars represent ± SE of means.

Results have showed that the non-grafted seedlings {Ratam (10.83 mm) and Pado (11.39 mm)} had similar rootstock diameter as observed from the seedling of the grafted variety (11.82 mm) (Figure 4a). Significant differences were exhibited among the interactive effect of scion and rootstock combinations on the diameter of the scion. The diameter of the scion ranged from 5.55 to 7.41 mm producing maximum from graft combination of Sindhri and Pado rootstock (Figure 4b). The lowest diameter of the scion (5.55 mm) was observed from the graft combination of Chaunsa variety with Pado rootstock. However, this chaunsa variety had good results when grafted with Ratam rootstock. Overall, Sindhri had good results with Ratam as well as with Pado rootstocks as compared to other scion and rootstock combinations.

Results of stionic ratio of grafting showed significant variation in response to various scion and rootstock combinations. On the basis of interactive effect of scion and rootstock combinations, maximum stionic ratio (0.93) was observed from the graft when Neelum variety was grafted on Pado rootstock. This Pado rootstock had also statistically similar results (0.89) with the Sindhri variety. Vice versa Sindhri as rootstock had poor results with each scion variety that ranges from 0.64 to 0.70 stionic ratio. The Pado rootstock had also lower results with Chaunsa variety. The best rootstocks both non-grafted Pado and Ratam had good results with Neelum variety (Figure 5a). The various scion varieties had significant effect on the leaves per graft. Neelum produced more number of leaves (16.33) as compared to Sindhri (9.89) and Chaunsa (5.00) (Figure 5b). Sturdiness quotient was significantly affected by the seed source. However, the seeds of each seed source produced seedlings with sturdiness quotient not above than 6. The data in Figure 5c showed that the seeds of the non-grafted Pado had better sturdiness quotient (3.89) than rest of the seed sources followed by Ratam (4.22) and grafted Sindhri (5.24)

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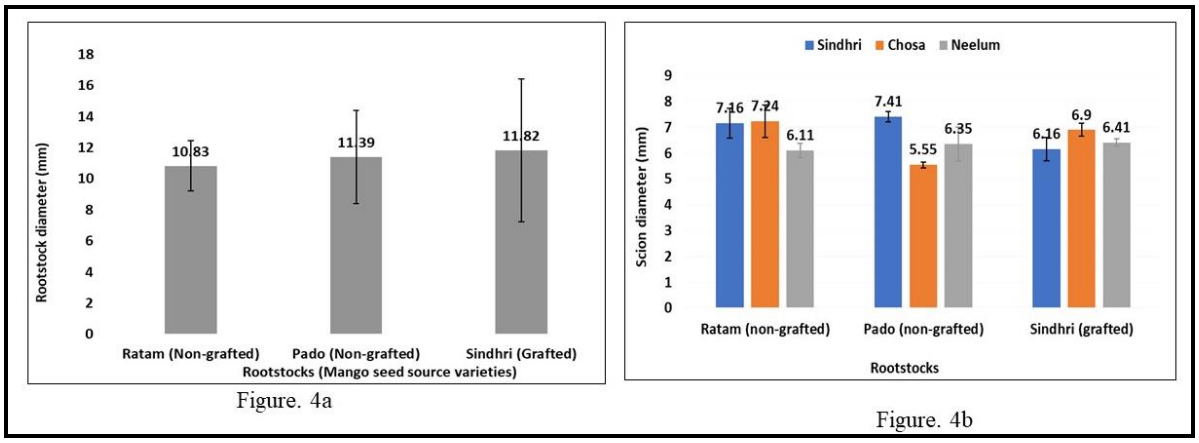

Figure 4. Performance of rootstock and scion diameter of mango varieties. Different letters indicate significant differences at $p \leq 0.05$. Vertical bars represent ± SE of means

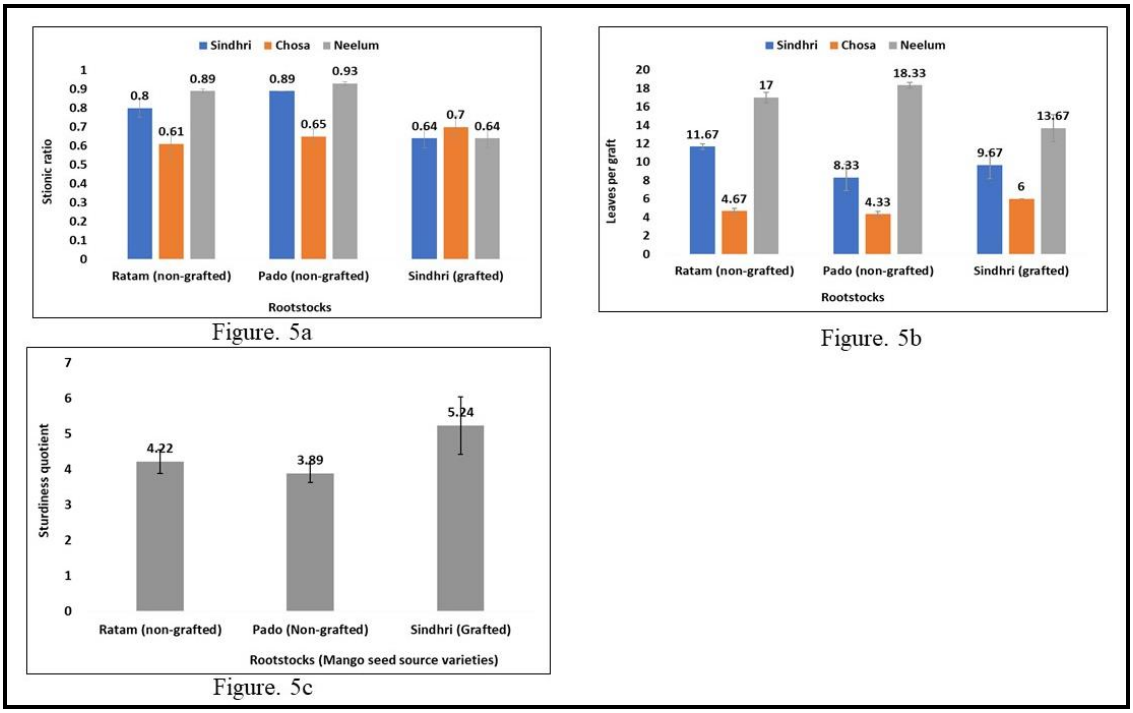

Figure 5. Performance of various physiological characters of mango varieties. Different letters indicate significant differences at $p \leq 0.05$. Vertical bars represent ± SE of means

DISCUSSION

In mango, seed germination plays an important role in producing the rootstock seedlings required for production of grafts. Minja *et al*., (2017) reported Ngwangwa with more vigorous rootstocks due to the large leaf area, number of leaves and length as compared to the Sindano and Zizi rootstocks. In addition to this they also reported Ngwangwa seedlings had longer leaves as compared to the Sindano and Zizi rootstocks. Prajapati *et al*., (2012) also observed similar results whereby change in characteristics such as plant height and number of leaves between several rootstocks prior to grafting was observed. Chandan *et al*., (2006) recorded more branches and leaves in Bappakai rootstock and the lowest number of leaves in local rootstocks. Chandan *et al*. (2006) also reported that height of Dashehari on different polyembryonic varieties had a positive correlation with stem girth and leaves. Seedling vigor is a complex physiological trial that is necessary to ensure the rapid and uniform emergence of plants in the field, essentially including the seed longevity, the tolerance of environmental stresses by germination (Ventura el al., 2012). Findings about chlorophyll content was obtained and reported by Chandan *et al*., (2006). They determined more chlorophyll content in leaves (1.44 mg g^{-1}) of Dashehari on Ec 95862 rootstock than all other rootstocks. The lowest chlorophyll in leaf was recorded in local rootstock (1.10 mg g^{-1}). The results were on par in Starch and Kitchner rootstock having respective values of chlorophyll content as 1.15 mg g^{-1} and1.16 mg g^{-1}. However, it is very important to clear that seedlings had more chlorophyll content before grafting. Genetic factors are being strongly associated with the rootstock and scion diameter of mango. Chandan *et al*., (2006) observed superior diameter of scion and rootstock combination of Dashehari variety with Bappakai rootstock. Hossain *et al*., (2008) reported that the diameter of scion was highly influenced by the different stionic relationship from 30 to 150 days after planting the grafts in the field. The plants produced by grafting at different stionic relationship showed differences in diameter of the scion. The highest diameter (0.96 cm) of the scion was observed in the plants produced by grafting the rambutan scion onto the rambutan rootstock. The appropriate stionic relationship and to obtain true-to-type planting materials is pre-requisite for strengthening the mango industry. Botanically those Plants belongs to similar family, they pretend higher success rates for graft union (Hartmann *et al*., 2011). Various factors may influence on graft success mainly physiological, grafting time and Climatic factors are prominent responsible for graft success. Besides, other factors such as rootstock identity and selection, grafting time and techniques also have great influence on success and survivability of grafts. Chandan *et al*., (2006) reported that the results of stionic compatibility on the basis of ratio (0.74) of scion and stock was found in Dashehari grafted on Ec 95862 rootstock and higher ratio (0.98) was found in Dashehari on Bappaki.

CONCLUSION

Results depict significant level of variations among rootstock and scion varieties of mango in stionic establishment of the seedlings. It is concluded

that Pado rootstock was observed better in terms of seedling rootstock and for successful stionic establishment of the mango seedlings.

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