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Standardization of vegetative propagation technique of wild edible Himalayas pear (*Pyrus pashia*) on newly identified local rootstock, RC Sohjhur-3

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ABSTRACT

*Pyrus pashia is an important underutilized fruit crop in Meghalaya, North East, India, having several ethnobotanical and pomological uses. However, the lack of quality planting materials and long juvenility of seedlings lead to their gradual population degradation. Therefore, a study was conducted to develop a protocol for vegetative propagation method of quality planting materials using RC Sohjhur-3 (IC-0632369) as the local rootstock. Two grafting methods (Tongue and Wedge grafting) in four different periods (15th of August, September, October and November) were used. The result showed a significant effect of the interaction of Wedge grafting *15th August on the least number of days taken to sprouting (10.4±0.3 days). However, maximum graft success (93.3±9.9%), survivability (86.7±4.9%), and growth performance of grafted plants were found in Tongue grafting *15th October. Therefore, Tongue grafting carried out on 15th October showed promising graft performance, which may help in the rapid production of quality planting materials for better utilization and conservation of this species.*

***Keywords*:** Fruit, graft success %, rootstock, tongue grafting, wedge grafting

Pyrus pashia is commonly known as the wild Himalayan pear, locally also called as *Sohjhur* and *Sohaitsyiar* in Khasi and Pnar, respectively in Meghalaya. The species is a member of the Rosaceae family with Himalayas encompassed the north east India as the centre of its origin. It is one of the most commonly underutilized fruits of the hill populace of the region (Rymbai *et al.,* 2019). The fruit tree is scattered throughout the Himalayan region's hill tracks, that encircle the north-eastern states of India, and extend from Pakistan to Vietnam as well as from the southern province of China to the northern region of India (Krause *et al*., 2007). Furthermore, its occurrence in Bhutan was found only in the backyards of small-scale farmers, and Nuristan in Afghanistan represented its furthest western distribution. In Meghalaya, this fruit tree is commonly seen throughout the state, where it is well-known and valued for its astringent taste and grittiness (Rymbai *et al*., 2019). The fruit morphology like; fruit length renged from 19.81 to 45.02 mm, diameter from 22.19 to 52.89 mm; volume from 3.40 to 66.40 cc, weight from 5.69 to 71.21 g, pulp weight from 3.80 to 42.96 g and seed weight from 0.34 to 0.57 g. Biochemical properties of fruits contain 6.02-11.82% total soluble solids, 0.27-0.40% titratable acidity, 14.94-41.62 TSS: Titratable acidity with peel colour (i.e., *a** value, 9.74 - 7.54 in shoulder; 10.24 - 18.27 in middle; 9.53- 7.28 in the apex portion of fruit) (Rymbai *et al*., 2016). This fruit is notably rich in protein, total solids, and sugar for consumption (Parmar and Kaushal, 1982). The fruit had an eye basin and a gritty pulp texture with a strong astringency and edible peel (Rymbai *et al*., 2016; 2019). Seedlings of *P. pashia* are used as rootstocks; leaf extract is used as a tonic for hair loss and the treatment of digestion-related ailments; it possesses antimicrobial activity. In addition, warm leaves extract is also consumed as beverages (Gulia, 2005). Therefore, the inclusion of this fruit may therefore improve the diet nutritionally and aid in preventing the various degenerative diseases due to the total anti-oxidant ability of fruit, which is the consequence of a coupling between the various bioactive compounds and radical oxygen species scavenger precursors (Rymbai *et al.,* 2013). In view of the prices and demand for mature fruits of this crop in the local market (Rs. 15–40 kg^{-1}) of Shillong and other parts of Meghalaya, it could be said that this underutilized crop is potentially a future crop (Rymbai *et al.*, 2019). The grafting operations under open field conditions are associated with several problems which hindered the graft success and survivability of plantlets, thus, reduced the rapid multiplication and shortage of quality planting materials (Rymbai and Reddy, 2010a).

Short Communication

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Polyhouse nursery settings have recently been recognised as a viable option to open field conditions for improving the effectiveness of nursery operations and field establishment of grafted plants (Rymbai and Reddy, 2010b). Therefore, this fruit tree's compatibility as a rootstock and its highly nutritious and aromatic fruits may provide additional income and nutrition to the inhabitants of the Himalayan hills, including the North East Regions of India. Therefore, a research was conducted to identify a suitable method and time of grafting for rapid multiplication of *P. pashia* which will facilitate its potential utilization and conservation.

The experiment was conducted at the Horticulture Farm, ICAR Research Complex for NEH Region, Umiam, Meghalaya, India which is situated at $25^0$41'91' North (latitude) and $91^0$55'15' East (longitude) with an altitude of 960 m above mean sea level during 2018-21. About 90% of the total annual precipitation (2684 mm) occurs during May–October. The mean temperature was recorded at the minimum (6.6 °C) during January and the maximum in August (29.06 °C). The minimum relative humidity was recorded during the winter (51.1%) and the maximum in the monsoon season (90.13%) .

The seeds were collected from a healthy mother plant of RC Sohjhur-3 (IC-0632369, a newly identified rootstock) for raising rootstocks. The seeds were thoroughly rinsed under running water and then subjected to stratification for about 2 weeks (at about 4–8 °C). The stratified seeds were sown in nursery bed during August. The seedlings were transplanted into polybags (comprising soil: sand: farmyard manure, 1:1:1 v/v) during January-February, when they attained a height of 6–8 cm and had 4–6 leaves. The one-year-old rootstocks (planted in polybags) of uniform size were used for grafting. Eight to ten months old shoot of the previous season were chosen from vigorous and elite mother plants as scion material. A scion shoots of size, 5–8 mm thick and 20–25 cm length containing 3 to 4 healthy and plumpy buds were used. Two grafting methods were used for the experiments, *viz*., 1) Tongue grafting and 2) Wedge grafting in four different periods, *viz*., 1 - 15th August, 2 - 15th September, 3 - 15th October and 4 - 15th November. The experiment was carried out under low-cost polyhouse conditions. Data on days taken to sprouting of grafted plants were recorded daily till 40 days after grafting (DAG). The graft success (i.e., initial sprouting of scion %) and survivability (%) of grafted plants were determined at 30 DAG and 150 DAG, respectively. Plant dimension (diameter of scion in mm; height of plant in cm), number of branches and leaves were measured at 30, 90 and 150 DAG.

$$\text{Graft success } (\%) = \frac{\text{Number of grafted plants sprouted at 30 DAG}}{\text{Number of plants grafted}} \times 100$$

$$\text{Survivability } (\%) = \frac{\text{Number of plants survived at 150 DAG}}{\text{Total number of plants grafted}} \times 100$$

A factorial randomised design with five replications, consisting of ten plants in each replication, was used. The statistical tool SPSS ver. 26 was used to perform a "Tukey Test" ($P<0.05$) on the differences between mean values.

With regards to sprouting and survivability behavior (Fig. 1 & 3; Table 2) of grafted plants, tongue grafting had minimum days taken to sprouting (17.74±0.5 days), however, had maximum graft success (85.56±6.6%) and survivability (75.0±5.2%). Grafting during the 15th August had the minimum days to sprouting (12.34±0.2 days) and graft success (73.3±4.7%). However, 15th October showed maximum days to sprouting (26.76±0.4 days) with the highest graft success (87.8±5.2%) and survivability (75.0±3.8%) (Fig. 3). The interaction of Tongue grafting and 15th August had the least days taken to sprouting (10.4±0.3 days) and graft success (68.9±9.3%). While, tongue grafting *15th October had the maximum graft success (93.3±9.9%) and survivability (86.7±4.9%). It indicated that tongue grafting shortened the sprouting period by 23.9% but enhanced the graft success by 11.6%, survivability (9.7%) and other scion and growth attributes as compared to wedge grafting. The graft success (19.7%), survivability (38.9%) and other scion and foliage growth were higher in the operation carried out during the 15th October. The differences in days taken of scion to sprouting might be due to the minimum time required by scion to get compatible with rootstock. Furthermore, the contact area of scion and stock was doubled in tongue grafting as compared to wedge grafts, thus facilitated better cambium union and vascular tissue development in graft union between scion and stock in the post operation. The higher tissue regeneration ensures a good transportation of water, nutrients and growth hormones (Hussain *et al*., 2016), thus, leading to higher plantlet development in tongue grafting over wedge grafting method. Our finding is in consistent with the report of Rymbai *et al*. (2012) in guava. It was also noted that the graft success and survivability were also increased by

Table 1: Multivariance ANOVA tests between-subjects' effects of methods, time of grafting and their interactions

Source	Methods					Periods					Methods x Period				
Dependent variable	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**	**Type III Sum of Square**	**df**	**Mean Squares**	**F**	**Sig.**
Days to sprouting	179.8	1	179.8	299.9	0.000	1179.8	3	393.3	656.0	0.000	30.6	3	10.2	17.0	0.000
Graft success (%)	789.9	1	790.0	13.8	0.001	1110.9	3	370.3	6.5	0.001	24.7	3	8.2	0.1	0.933
Survivability (%)	444.2	1	444.4	16.9	0.000	2876.2	3	958.7	36.5	0.000	24.7	3	6.7	0.3	0.015
Scion diameter															
30 DAG	0.15	1	0.2	3.1	0.086	0.6	3	0.2	4.1	0.015	0.0	3	0.0	0.1	0.966
90 DAG	1.9	1	1.9	10.7	0.003	4.2	3	1.4	7.8	0.000	0.1	3	0.1	0.3	0.844
150 DAG	6.0	1	6.0	26.8	0.000	17.9	3	6.0	26.5	0.000	1.0	3	0.4	1.6	0.022
Scion height															
30 DAG	0.5	1	0.6	0.1	0.730	11.5	3	3.8	0.8	0.501	0.6	3	0.2	0.0	0.987
90 DAG	62.7	1	62.7	12.6	0.001	275.0	3	91.7	18.4	0.000	12.4	3	4.2	0.8	0.049
150 DAG	318.4	1	318.4	36.0	0.000	270.7	3	90.3	10.2	0.000	7.4	3	2.5	0.3	0.024
Number of branches															
30 DAG	0.2	1	0.3	27.0	0.000	0.3	3	0.1	12.1	0.000	0.0	3	0.0	1.4	0.260
90 DAG	0.5	1	0.5	22.5	0.000	1.7	3	0.6	24.9	0.000	0.0	3	0.0	0.6	0.599
150 DAG	3.3	1	3.4	110.3	0.000	5.3	3	1.8	57.9	0.000	0.4	3	0.1	3.9	0.018
Number of leaves															
30 DAG	6.2	1	6.2	249.0	0.000	9.5	3	3.2	127.5	0.000	0.4	3	0.1	5.5	0.004
90 DAG	422.5	1	422.5	1356.3	0.000	495.5	3	165.2	530.2	0.000	5.0	3	1.7	5.4	0.004
150 DAG	746.5	1	746.5	332.4	0.000	1336.4	3	445.5	198.4	0.000	114.4	3	38.2	17.0	0.000

DAG indicates days after grafting

Table 2: Effect of the interaction of methods and time of grafting on sprouting, graft success and growth parameters of *Pyrus pashia*

Treatments	Days to sprouting	Graft success (%)	Survivability (%)	Scion diameter (mm)			Scion height (cm)			Number of branches			Number of leaves		
				30 DAG	90 DAG	150 DAG	30 DAG	90 DAG	150 DAG	30 DAG	90 DAG	150 DAG	30 DAG	90 DAG	150 DAG
TG*M1	10.4±0.3^{g}	77.8±7.8^{bc}	64.5±4.9^{cd}	5.5±0.1^{a}	5.8±0.1^{bc}	6.1±0.3^{d}	31.9±1.7^{a}	34.8±1.6^{bc}	42.6±1.5^{bc}	0.24±0.09^{cd}	1.3±0.2^{bcd}	1.9±0.2^{cde}	2.2±0.1^{cd}	12.8±0.1^{d}	34.5±1.8^{ef}
TG*M2	21.4±0.9^{c}	86.7±4.9^{ab}	77.8±0.0^{ab}	5.2±0.3^{a}	6.2±0.1^{abc}	7.3±0.1^{abc}	31.6±2.1^{a}	37.3±1.3^{bc}	45.5±2.0^{ab}	0.36±0.09^{bc}	1.5±0.1^{bc}	2.4±0.1^{b}	2.5±0.2^{bc}	18.9±0.6^{b}	43.6±0.6^{b}
TG*M3	23.2±1.5^{b}	93.3±9.9^{a}	86.7±4.9^{a}	5.5±0.2^{a}	6.7±0.2^{a}	8.2±0.2^{a}	31.8±0.8^{a}	43.1±3.0^{a}	50.8±3.9^{a}	0.56±0.17^{a}	1.9±0.1^{a}	3.1±0.2^{a}	3.5±0.1^{a}	23.5±0.8^{a}	51.4±1.7^{a}
TG*M4	15.9±0.7^{e}	84.5±6.1^{ab}	71.1±6.0^{bc}	5.3±0.1^{a}	6.2±0.4^{ab}	7.1±1.1^{bc}	30.5±4.5^{a}	36.6±0.9^{bc}	46.1±3.9^{ab}	0.44±0.09^{ab}	1.4±0.2^{bcd}	2.2±0.3^{bcd}	2.3±0.3^{bc}	16.4±0.6^{c}	40.3±1.5^{cd}
WG*M1	14.2±0.4^{f}	68.9±9.3^{c}	55.6±7.8^{d}	5.1±0.4^{a}	5.3±0.9^{c}	5.8±0.2^{d}	31.3±1.1^{a}	32.6±1.5^{c}	37.4±3.2^{c}	0.16±0.09^{d}	1.1±0.1^{d}	1.5±0.1^{f}	1.2±0.1^{f}	7.4±0.5^{f}	24.9±1.2^{g}
WG*M2	24.2±0.7^{b}	80.0±4.9^{abc}	71.1±6.1^{bc}	5.2±0.1^{a}	5.7±0.4^{bc}	6.1±0.1^{d}	31.4±1.2^{a}	35.3±2.2^{bc}	41.0±2.2^{bc}	0.24±0.09^{cd}	1.3±0.1^{cd}	1.8±0.2^{de}	1.6±0.2^{e}	12.1±0.4^{d}	32.3±1.0^{f}
WG*M3	30.4±0.3^{a}	82.2±6.1^{abc}	80.0±4.9^{ab}	5.4±0.3^{a}	6.2±0.1^{abc}	7.4±0.5^{ab}	31.8±0.6^{a}	38.7±2.9^{bc}	43.9±3.4^{b}	0.36±0.09^{bc}	1.6±0.2^{b}	2.2±0.2^{bc}	2.6±0.1^{b}	16.0±0.5^{c}	40.6±1.5^{bc}
WG*M4	19.2±0.5^{d}	75.6±9.3^{bc}	66.7±0.0^{c}	5.3±0.1^{a}	6.0±0.2^{abc}	6.4±0.4^{cd}	30.4±2.5^{a}	35.2±3.1^{bc}	40.2±2.5^{bc}	0.20±0.00^{cd}	1.2±0.3^{cd}	1.7±0.1^{ef}	1.9±0.3^{de}	10.1±0.2^{e}	37.4±2.1^{de}

Mean value (n = 30) with ±S.E followed by different letters on each bar indicate significant difference from each other according to Tukey's test ($p < 0.05$). (TG – tongue grafting; WG – wedge grafting; M1 – 15th August; M2 – 15th September; M3 – 15th October; M4 – 15th November)

19.7% and 38.8%, respectively in 15th October as compared to other period of operations. However, 15th August had early sprouting as compared to other grafting. The quick sprouting of grafted plants in the 15th August might be due to optimum temperature and day light. A similar effect has also been reported in rapeseed (Afshari *et al.,* 2011). The scion materials obtained during 15th October had sufficient carbohydrate content for sprouting and growth performance. The fruiting phenology of *P. pashia* indicates that the fruits attained maturity during July and completed the harvesting by 2nd week September. While, the bud burst of shoots occurred during 1st-2nd week November. Therefore, the lower percentage of success graft took during this period (15th August – 15th September) might be due to immature budwood, low sap flow and exhausted food reserved in the shoots. While, the scion shoots collected during 15th October might have sufficient period of accumulation for higher starch content as compared to early (i.e., on and / or immediately after harvesting during 15th August-15th September) and late (post bud burst and sprouting during 15th November) scion shoots. These phenomena might have enhanced the cambium union of scion-stock relationship in *P. pashia* on local rootstock and their growth performance. A quantitative alteration in starches that enables the scion to form a graft union may also be related to the seasonal variation in grafts (Martínez-Ballesta *et al*., 2010). Salik *et al.* (2015) also noted the impact of grafting duration on scion development in orange under arid zones.

A significant effect of methods, periods of grafting and their interaction on scion dimension attributes was observed (Fig. 2a-b & 4a-b; Table 2). Regarding methods of grafting, there were no significant differences between tongue and wedge grafting on scion diameter and scion height at 30 DAG. However, tongue grafting had significantly higher scion diameter than wedge grafting at 90 DAG (6.23±0.05 mm) and 150 DAG (7.2±0.08 mm). Similarly, the scion height was maximum in tongue grafting at 90 DAG (37.9±0.35 cm) and 150 DAG (45.2±1.05 cm). The months of grafting exhibited non-significant effect on scion diameter and height at 30 DAG. However, significant differences were noticed for scion attributes at 90 DAG (diameter, 40.9±1.75 cm; height, 1.76±0.37 cm) and 150 DAG (scion diameter, 47.1±5.33 cm; scion height, 2.68±0.07 cm) which was recorded the highest in 15th October over other period of grafting. The interaction effect of grating methods *periods also had no significant on scion diameter at 30 DAG. While, the scion diameter was significantly higher in TG *M3 at 90 DAG (6.7±0.2 mm) and 150 DAG (8.2±0.2) over other interactions. The

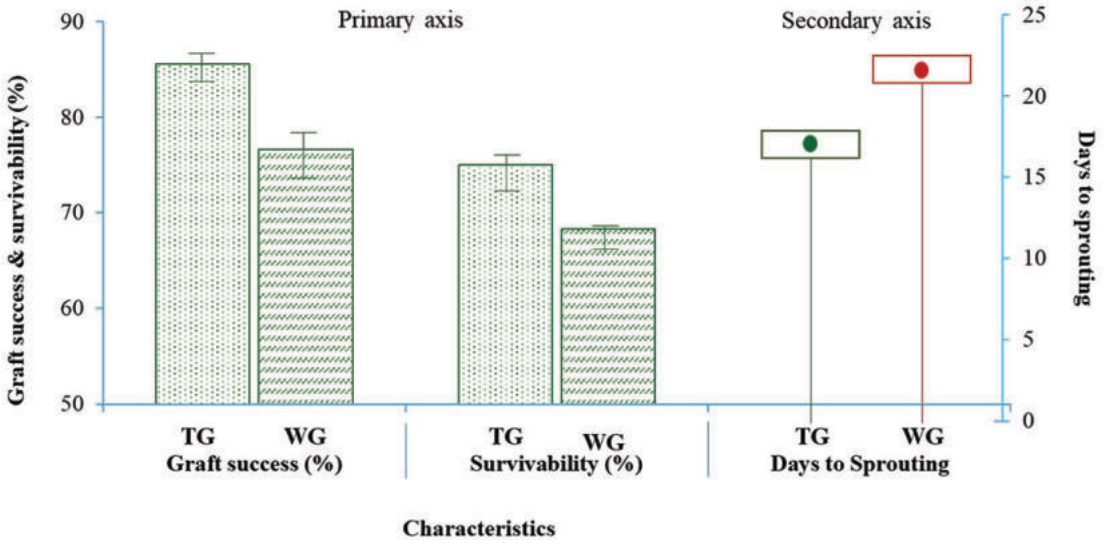

Fig. 1: Effect of grafting methods on sprouting and survivability of *P. pashia* grafted plants (TG – tongue grafting; WG – wedge grafting).

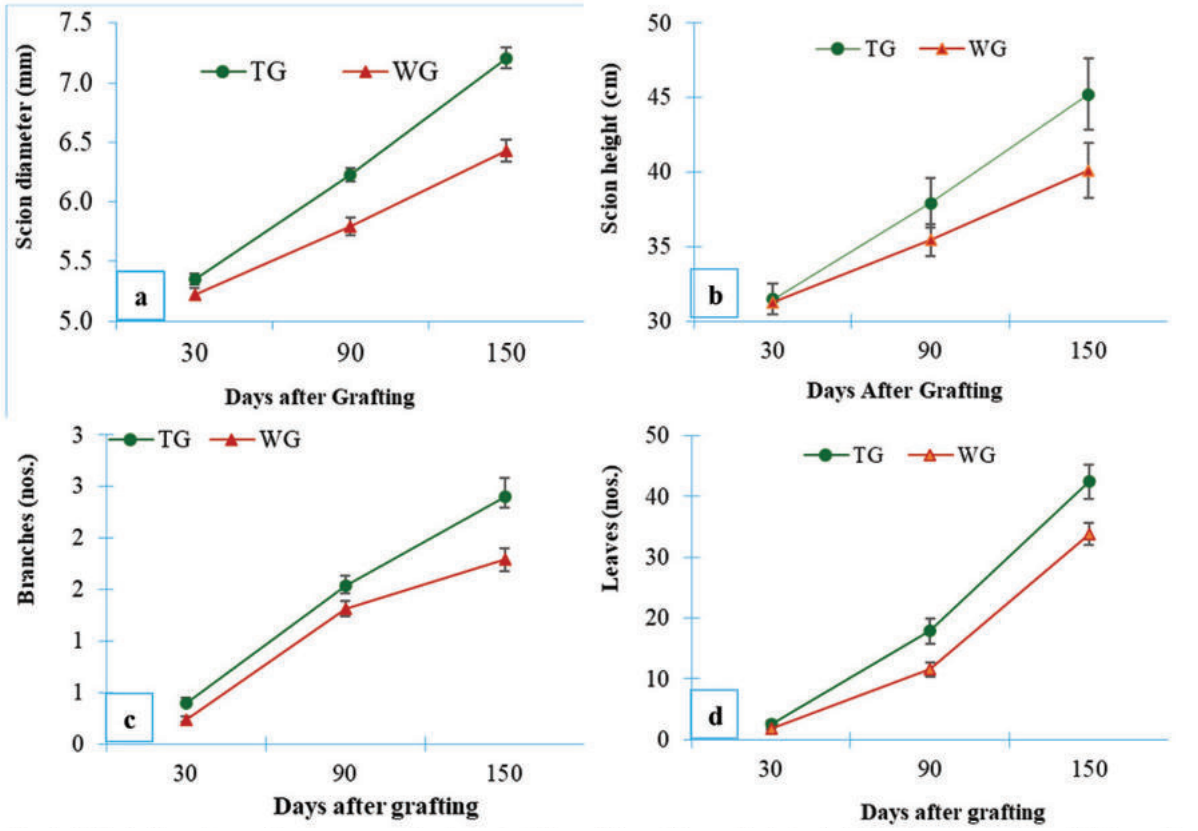

Fig. 2: Effect of grafting methods on growth characteristics of *P. pashia* grafted plants (TG - tongue grafting; WG - wedge grafting)

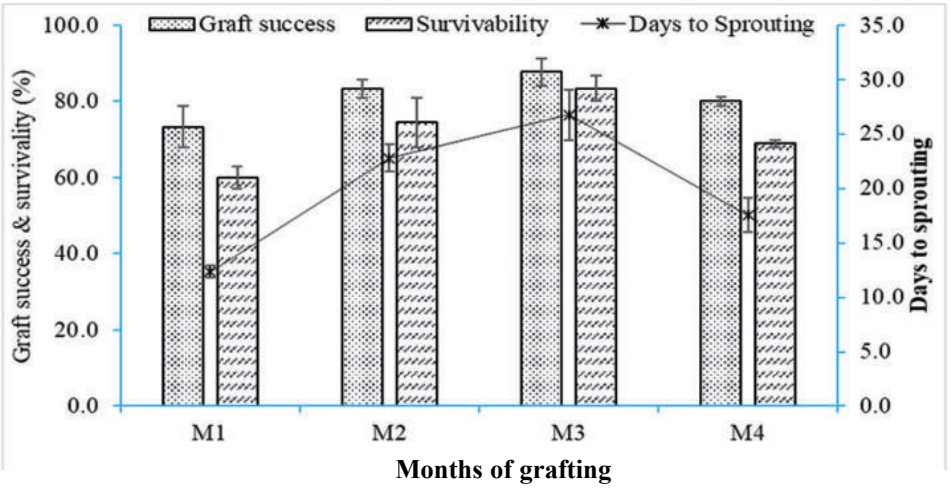

Fig. 3: Effect of period of grafting on sprouting and survivability of *Pyrus pashia* grafted plants (M1 - 15th August; M2 - 15th September; M3 - 15th October; M4 - 15th November)

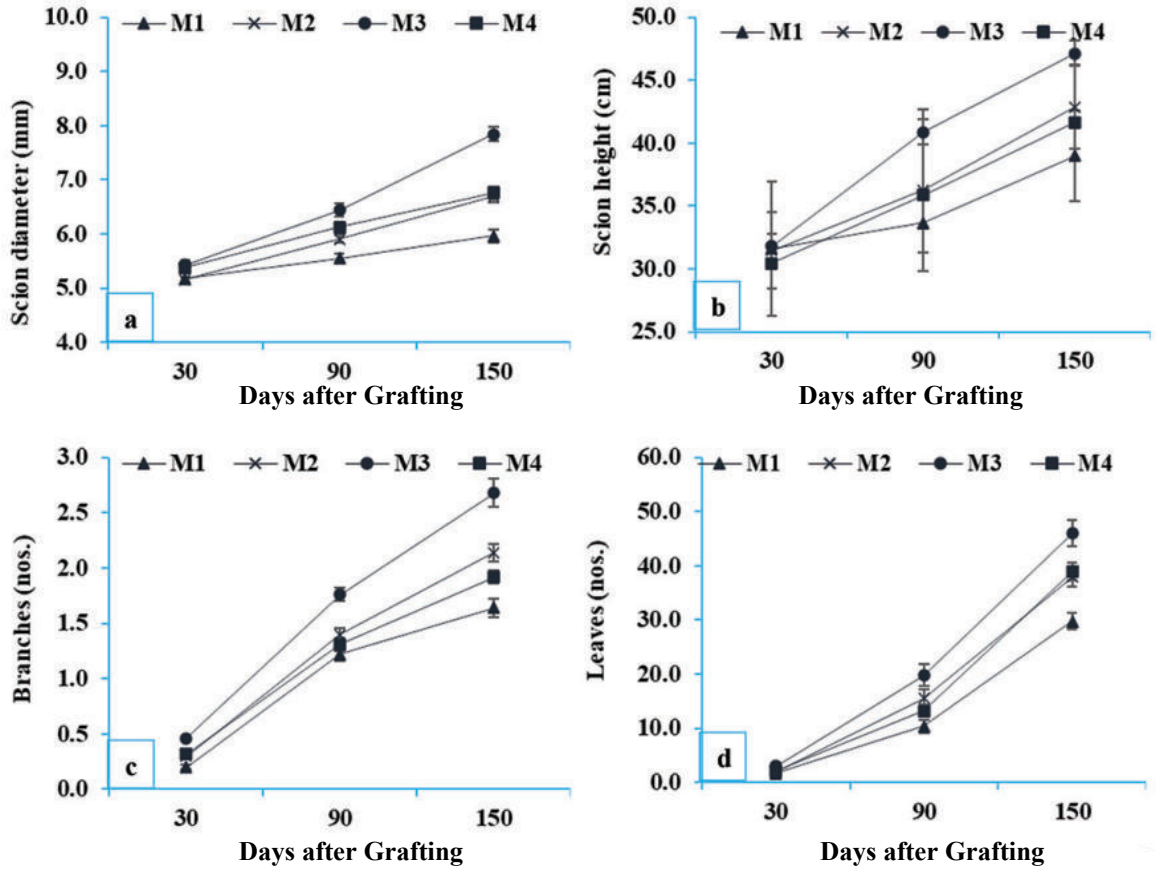

Fig. 4: Effect of months of grafting on growth characteristics of *Pyrus pashia* grafted plants (M1 - 15th August; M2 - 15th September; M3 - 15th October; M4 - 15th November)

higher scion diameter and scion height from 90 DAG onwards due to Tongue grafting *15th October might be due to a higher tissue regeneration of graft union which enhanced the transportation of water, nutrients and growth hormones (Hussain *et al*., 2016). Furthermore, the higher scion dimension in Tongue grafting *15th October might be due to a higher graft took and survivability of grafted plants (Fig. 1 & 3) as reported in *Antidesma bunius* L. (Rymbai *et al.,* 2022) and *Anacardium occidentale* (Praveen *et al*., 2022).

There was a significant different on branching patterns in *P. pashia* due to methods and periods of grafting, and their interactions (Fig. 2c & 4c; Table 2). Regarding methods of grafting, tongue grafting had higher number of branches over wedge grafting at 30 DAG (0.40±0.03 number per plantlet), 90 DAG (1.54±0.07 number per plantlet) and 150 DAG (2.5±0.42 number per plantlet). Among period of grafting, the 15th October had the highest number of branches at 30 DAG (0.46±0.07 number per plantlet), 90 DAG (1.76±0.11 number per plantlet) and 150 DAG (2.68±0.25 number per plantlet). The interaction effect of grafting methods and timing showed that tongue grafting *15th October produced maximum number of branches over other periods at 30 DAG (0.56±0.17 0.46 number per plantlet), 90 DAG (1.9±0.1 number per plantlet) and 150 DAG (3.1±0.2 number per plantlet). The higher number of branches in Tongue grafting * 15th October might be due to a higher scion diameter and height as reported by Mngomba *et al*. (2012) on the positive effect of scion and stock diameter on number of branches and growth of *Sclerocarya birrea*.

The grafting methods, times, and their interactions had significantly affected the foliage attributes as shown in Fig. 2d & 4d; Table 2. The highest number of leaves was noted in tongue grafting throughout the growth periods, *i.e*., 30 DAG (2.61±0.02 number per plantlet), 90 DAG (17.91±2.3 number per plantlet) and 150 DAG (42.47±3.02 number per plantlet) over wedge grafting. Among periods of grafting, the 15th October showed a noticeably increase in leave number at 30 DAG (3.0±0.05 number plantlet^{-1}), 90 DAG (19.8±1.63 number plantlet^{-1}) and 150 DAG (46.0±2.84 number plantlet^{-1}) over other months of grafting. Among the interaction effect of method and time of grafting, TG*M3 was found to significantly increased the number of leaves at 30 DAG (3.5±0.1 number per plantlet), 90 DAG (23.5±0.8 number per plantlet) and 150 DAG (51.4±1.7 number per plantlet). Tongue grafting * 15th October increased the foliage attributes might be due to higher number of branches. It was also reported by Gurung *et al.* (2020) in *Citrus reticulata* and Rymbai *et al*. (2022) in *Antidesma bunius* that a higher number of leaves was associated with higher number of branches and shoot performance in vegetatively propagated plants.

It is concluded that tongue grafting carried out during the 15th October produced the highest graft success, survivability, scion dimension and growth performance in *Pyrus pashia* on local rootstock, RC Sohjhur - 3. Therefore, this vegetative propagation technique standardization will help in production of quality planting materials for effectively utilization and conservation of *P. pashia* which is an important underutilized fruit crop in North East India.

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