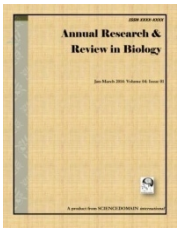

Annual Research & Review in Biology
4(14): 2337-2346, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

In vitro Grafting of Selected Papaya (*Carica Papaya* L.) Lines in Kenya

Naomi Nzilani Mumo[1*], Fredah Karambu Rimberia[1], George Edward Mamati[1] and Agnes Wanjiru Kihurani[1]

[1]Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.

Authors' contributions

This work was carried out in collaboration between all authors. Authors NNM, FKR and AWK developed the concept and designed the experiment. Authors NNM and GEM performed the statistical analysis and the interpreted the data. All authors wrote the protocol, read and approved the final manuscript.

Original Research Article

Received 13th February 2014
Accepted 12th March 2014
Published 5th April 2014

ABSTRACT

The aim of this study was to evaluate success of *In vitro* grafting method in three selected Kenyan papaya lines. In vitro regenerated shoots of about 20mm were used where the upper 10mm tips were excised and used as scions while the remaining portion was used as rootstock. The rootstocks and the scions were used interchangeably. Number of leaves and length of grafts were recorded every week for six weeks. Number of scions that were alive after six weeks was also recorded. Grafted shoots were rooted on MS with 2.5mg/l IBA. The number of grafted shoots that were alive after six weeks ranged between 45% and 80%. The types of rootstock and scions affected the number of leaves and length of grafted shoots with lines 1 and line 2 grafted on their rootstock exhibiting the highest leaf number and shoot length. Papaya line 3 grafted on either line 1 or 2 rootstock and vice versa had a higher number of leaves and shoot length as compared with line 3 grafted on its own rootstock. Within 24 weeks, in vitro grafted plantlets were achieved. In vitro grafting of selected papaya lines was successful.

Keywords: Carica papaya; In vitro grafting; lines; propagation; success.

**Corresponding author: Email: nzimumo@yahoo.com;*

1. INTRODUCTION

Papaya (*Carica papaya L.*) is a member of *Caricaceae* family. It is a valuable fruit crop grown commercially in many tropical and subtropical countries. Papaya is easy to cultivate and has good agronomic features such as rapid growth, requirement of minimal space, early maturation and high yields [1]. Papaya is also commonly grown in home gardens and scattered among other crops in smallholder plots due to its single stemmed nature.

Papaya ripe fruits are rich in vitamins A and C [2] and are commonly used as fresh dessert. It is also eaten raw, sliced into thin strips and eaten as vegetable or processed into various products such as candy, pickle or puree [1]. The latex from unripe fruit and leaves contains a proteolytic enzyme, papain, which is used for tenderizing meat, chill-proofing beer, tanning leather and for making chewing gum [3]. In pharmaceutics, papain is used for suppression of inflammation, treatment of gangrenous wounds and for various digestive ailments [4]. As a proteolytic enzyme, it has exfoliating property that removes the dead surface cells of the skin, giving it a rejuvenated feeling [1]. It is therefore popularly used in soaps, creams, shampoos and lotions in the cosmetic industry.

In Kenya, papaya is a widespread fruit crop throughout the country [5] grown by both the small and large scale farmers [6]. The major producing provinces are Coast, Nyanza, Western, Rift Valley, Eastern and Central provinces [7]. The fruit is grown for both local fresh consumption, processing and export markets, hence a good source of nutritious food and income to farmers.

Seed propagation is a common method of propagation in papaya. However seed propagation is hindered with problems associated with inherent heterozygosity and dioecious nature of the plant [8]. These features of the plant impose considerable limitations on improvement work and are some of the major reasons for the lack of true varieties in this important fruit crop [9]. Clonal methods for propagation can overcome some of these difficulties in papaya cultivation and improvement.

Asexual propagation methods in papaya such as grafting [10] and rooted cuttings [11] exist, but they are often not carried out on a large scale [12] because they are generally cumbersome, time consuming and highly season bound with low multiplication rates. Nevertheless, grafting in papaya has been attempted in an effort to address the problem of raising papaya plants with the desired sex [10, 13 and 14].However, bacterial infection of the scions [10] and soft rot fungi infection [14] has been reported to reduce the success rate of grafting. Grafting of disease-free explants is an appropriate alternative for propagation of papaya plant.

In vitro grafting is a well recognized propagation method for many plant species [15]. It consists of grafting, under aseptic conditions, a small shoot tip onto a young seedling or plantlet root stocks growing *In vitro* [16]. *In vitro* grafting has several unique uses including: production of disease-free plants by grafting small meristem tips [17], virus indexing by micro grafting to susceptible under stocks [18], early detection of grafting incompatibility relationships [19], propagation of novel plants created in tissue cultures that are difficult-to-root [20] and small micro grafted trees are a convenient way to exchange germplasm between countries [21].

In vitro grafting has potential to be utilized as alternative method of papaya propagation. However, to the best of my knowledge, there is no documented scientific research devoted

to *In vitro* grafting in papaya to date. Therefore, assessing *In vitro* grafting success of papaya would be necessary to evaluate is applicability for propagation of papaya.

2. MATERIALS AND METHODS

2.1 Study Site

The study was conducted in the Tissue Culture Laboratory of Institute for Biotechnology Research (IBR), Jomo Kenyatta University of Agriculture and Technology (JKUAT).

2.2 Plant Materials for Research

Fruits of three local adapted papaya lines from ongoing papaya research project in JKUAT, Juja, were selected based on their superior performance. Seeds were extracted and stock plants established in a green house. When the seedlings were three month old, 1.0cm shoot tips were excised and sterilized in 20% household bleach (jik®) containing 3.85% sodium hypochlorite and 2 drops Tween 20® for 10 minutes. Thereafter, the shoot tips were three times rinsed with sterilized distilled water.

After surface sterilization, *In vitro* regeneration method described by Mumo et al. [22] was used for shoot multiplication and elongation. Cactus thorns were harvested and washed in running tap water for about 30 minutes and sterilized by autoclaving at 121°C at 1.5Mpa pressure for 20 minutes and then stored in absolute ethanol until use.

Under aseptic conditions, rootstock and scion partners of similar diameter were selected to optimise potential cambial contact. For ease of working, the shoots were defoliated taking care not to damage the apex. Shoots of 20mm in length were used whereby the upper 10mm of the tips were excised and used as scion, while remaining portion was used as rootstock. Wedge grafting was used where vertical incisions were made on the top end of rootstocks. The bottom ends of shoots were cut into a wedge (“V”) shape and placed into the incision made in the rootstock with both the cut surfaces in good physical contact and then held together at the point of graft with sterile cactus thorns. Rootstocks and scions were used interchangeably.

Combination of scion and rootstock resulted in formation nine graft combinations viz.: Line 1/1, Line 2/2, Line 3/3, Line 1/2; Line 1/3; Line 2/1; Line 2/3; Line 3/1; Line 3/2.The experiment was set in a Completely Randomized Design with 4 replicates and each replicate with 10 grafts. Grafted shoots were cultured on Murashige and Skoog (MS) (23) medium supplemented with 0.1mg/l BAP combined with 0.05mg/l NAA, 30g/l sucrose and 2.5g/l gerlite. Cactus thorns were removed after 6 weeks and the grafted shoots were rooted using procedure described by Mumo et al. [22]. Cultures were placed in growth chamber at 25±1°C and 16 hour photoperiod lighting was provided by Philips light bulb tubes that provided white fluorescent light (40W) in the growth chamber.

Number of leaves and length (mm) of each graft was recorded every week for 6 weeks. The percentage survival of grafted shoots that were alive after 6 weeks was also recorded. Number of leaves, shoot length and percentage survival were subjected to ANOVA contrast to detect significant differences between grafts means and graft means that were significantly different were compared using student Newman Keuls (SNK) test at $p \leq 0.05$

2.3 Acclimatization

Rooted plantlets were taken out of the culture containers and washed carefully under running tap water for complete removal of the rooting medium. Plantlets were transferred on pots (9x6cm) filled with forest soil, manure, sand and vermiculite in the proportion of 2:1:1:1, respectively. Within 3 weeks in acclimatization chamber, plants had hardened and were taken in greenhouse for further growth. About 25% of *In vitro* grafted plants remained alive after acclimatization

3. RESULT

Three to seven days after grafting, callus was formed at the point of graft union (plate 1 A). New leaves were formed in all grafts between four and seven days after grafting (Plate 1 B).

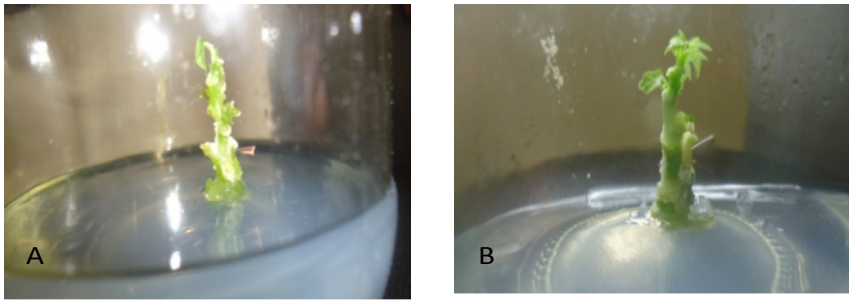

Plate 1. Callus formation at the graft union (A); formation of new leaves 4 to 7 days after grafting (B)

Increase in number of leaves was observed from week one to week 5 Fig. 1. There was no increase in number of leaves from week 5 to week 6 in all grafts. Significant influences of both rootstock and scions combinations on the mean number of leaves per grafts ($p<0.001$) were noted. Highest number of leaves was recorded in Line 1/1 and Line 2/2 while the least number of leaves was recorded in Line 3/3. Grafting Line 3/2, Line 3/1 Line 2/3, and Line 1/3 resulted into increased number of leaves compared to Line 3/3 while grafting Line 1/3, Line 1/2 and Line 3/1 and Line 2/1 resulted into reduction in number of leaves compared with Line 1/1. Comparing Line 2/2 with Line 1/2, Line 3/2, Line 2/1 and Line 2/3 resulted into reduced number of leaves Fig. 1.

There was increase in shoot length from week one up to week five. There was no increase in shoot length from week 5 to week 6 in all grafts Fig. 2. The results showed significant influences of both rootstock and scions combinations on the mean shoot length per grafts ($p<0.001$). Line 1/1 and Line 2/2 recorded the highest shoot length throughout the six weeks. The least mean shoot length was recorded in Line 3/3 Fig. 2. Grafting Line 3/2, Line 3/1 and Line 2/3, Line 1/3 resulted into increased shoot length compared to Line 3/3 while grafting Line 1/3, 1/2 and Line 3/1 and 2/1 resulted into reduction in shoot length compared with Line 1/1. Also comparing Line 2/2 with Line 1/2 and Line 3/2 and Line 2/1 and Line 2/3 resulted into reduced shoot length.

There were instances where graft union did not occur as witnessed when the scions and rootstock were poorly aligned (Plate 2A) and in other instances, scions necrosis and rootstock produced side shoots at the graft union (Plate 2B).

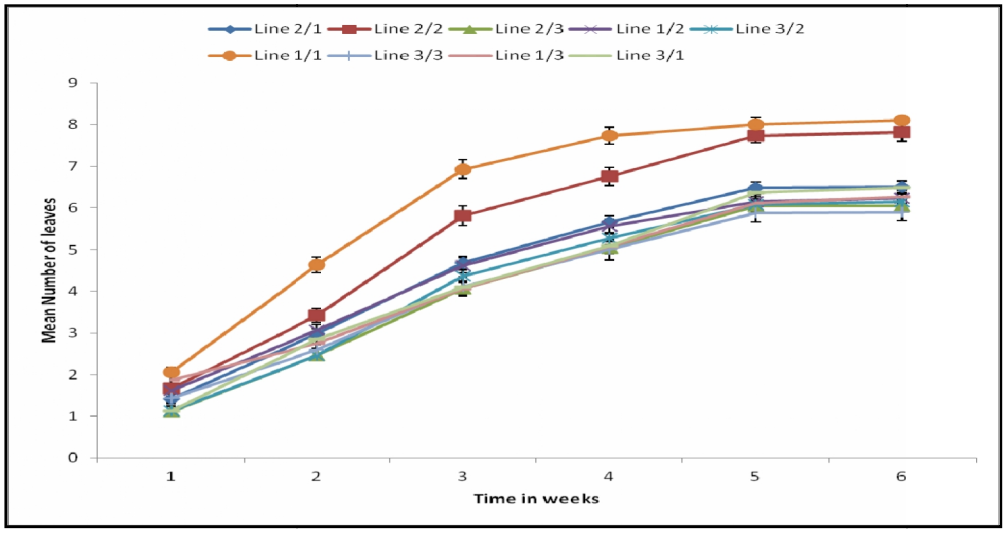

Fig. 1. Effect of grafts combinations on the mean number of leaves 6 weeks after grafting

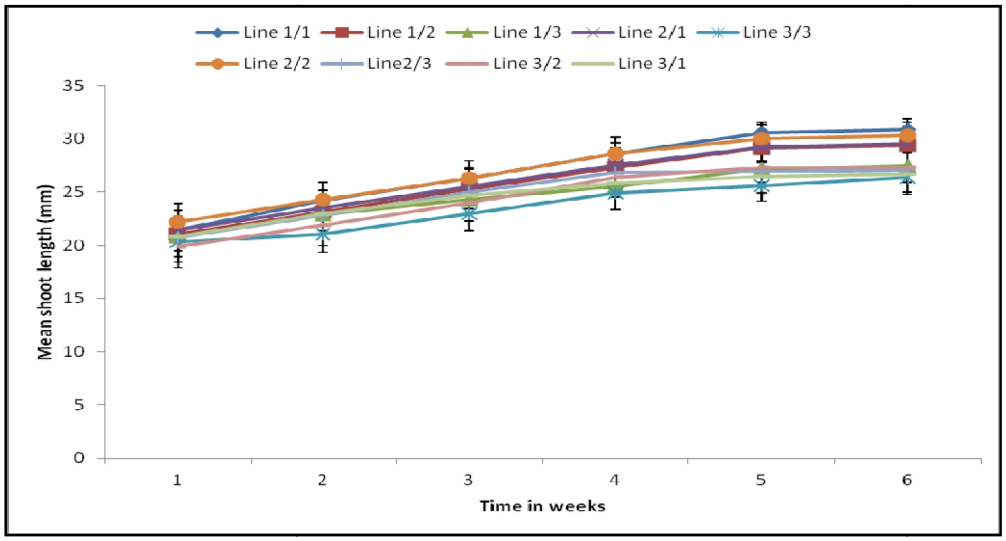

Fig. 2. Effect of graft combinations on average shoot length (mm) 6 weeks after grafting

After six weeks, the number of scions that were alive varied among the grafts Table 1. The highest number of scions that were alive was recorded in Line 2/2 with 80% followed by line 1/1 with 77.5% while the least was recorded in line 3/2 with 45%. No significant difference was noted on percentage successful grafts on Line 1/1, Line 2/2 and Line 3/3 Table 1.

Rooted in vitro grafted plantlets were achieved after 8 weeks in rooting media and plantlets ready for transferring into a greenhouse for further growth were achieved within 3 weeks after acclimatization (plate 3).

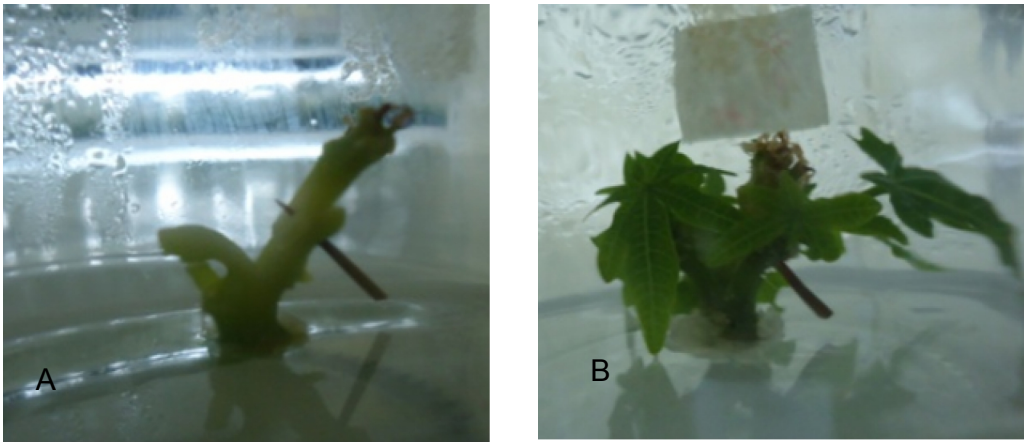

Plate 2. Graft failure due to poor alignment of scion and rootstock (A) necrosis of scions and production of side shoots by rootstock (B)

Table 1. The survival rate (%) of in vitro grafted shoots 6 weeks after grafting

Papaya line graft combinations	No. of shoots grafted	No. of grafted shoots alive	Survival rate of *In vitro* grafted shoots (%)
Line 1/1	40	30	77.5 ± 4.78^{a}
Line 2/1	40	28	70.0 ± 4.08^{a}
Line 3/1	40	20	50.0 ± 3.08^{cd}
Line 1/2	40	24	60.0 ± 4.08^{bcd}
Line 2/2	40	32	80.0 ± 4.08^{a}
Line 3/2	40	18	45.0 ± 5.00^{d}
Line 1/3	40	20	50.0 ± 2.88^{cd}
Line 2/3	40	19	47.5 ± 4.78^{cd}
Line 3/3	40	26	65.0 ± 2.88^{abc}

Mean values within a column followed by the same letter are not significantly different by SNK ($p \leq 0.05$*)*

Plate 3. Acclimatization of in vitro grafted plantlet

4. DISCUSSION

Establishing an in vitro grafting method of papaya may combine advantage of grafting with those of micropropagation. Seven days after grafting, calluses were formed at the graft union. Callus growth is a key process in the development of the graft union because it physically joins the scion to the rootstock [24]. It has been reported that grafting failure can be characterized by a lack of callus formation at the graft interface. Oda et al. [25] reported that low callus formation between the rootstock and scion could lead to defoliation, reduction of scion growth and low survival of grafted plants. Other studies have revealed that the new callus formed is a passive event that occurs in compatible and incompatible grafts and is a common response to wounding [26].

Dislocations of the grafts resulted in drying out of the scion and graft failure. The restoration of vascular continuity through the interface region is very crucial since it determines the compatibility between the rootstock and scion on the development of graft union formation [24 and 27]. This is because the restoration of the vascular bundles ensures flow of mineral nutrients and/or water between the rootstock and the scion [25 and 28]. Shoot necrosis was observed in some cases and formation of side shoots by the rootstock. Studies have reported that lack of, or decrease in the number of differentiated vascular bundles, or the dysfunction of differentiated vascular bundles at the graft union inhibit the transport of nutrients [29,30] leading to growth suppression of the scion and premature death.

The process of scion growth which occurred between 4-7 days was independent of graft success. It is possible that the initial development of scion occurred due to uptake of xylem sap exuded by decapitated rootstock plantlets [31]. However, the continued development of scion leaves and shoot length has been attributed to the functionality of regenerated vascular system [32] that allows translocation of water, mineral nutrition and carbohydrates [33] which are needed for production of new leaves and shoot growth.

No significant difference was noted on percentage successful grafts on Line 1/1, Line 2/2 and Line 3/3 and they had the highest percentage success compared with other graft combinations. This similarly, Rafail and Mosleh [34] when developing a protocol for in vitro shoot tip grafting for different cultivars of apples (MM106 and Anna) and pears (Aly-Sur and P. calleryana) reported highest micrografting success (90%) in grafting P. calleryana pear on P. calleryana stocks followed by 80% micrografting success in case of the grafting of MM106 apple scions on MM106 apple stocks which were significantly higher than the grafting of heterografting between different cultivars.

Given that the factors that influence plant growth (light, nutrition and temperature) represented a uniform treatment in the experiment, growth differences in grafted papaya plants indicate the effect rootstock and scion combinations on number of leaves and shoot length as well as percentage number of scions that were alive after six weeks.

It is interesting that the papaya lines 1 and 2 grafted on their rootstock showed vigorous growth in terms of number of leaves and shoot length. However, when line 1 was grafted on line 2 and vice versa, the growth was not vigorous as compared to Line 1/1 and line 2/2. This suggests that the total growth of grafted plants may not be directly related to the relative growth rate of either the rootstock or the scion.

5. CONCLUSION

From this study of *In vitro* grafting of selected papaya lines, the technique was successful and could be applied as an alternative method of propagating true-to-type papaya lines thereby overcoming conventional papaya propagation problems.

ACKNOWLEDGEMENT

The authors wish to thank the Regional University Forum (Ruforum) for providing funds to carry out this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Chan YK. Breeding Papaya (*Carica papaya* L.). S.M. Jain, P.M. Priyadarshan (eds.), Breeding Plantation Tree Crops: Tropical Species, C_Springer Science+Business Media, LLC; 2009.
2. Farzana ARF, Palkadapala PG, Meddegoda KM, Samarajeewa PK, Easwara JP. Somatic embryogenesis in papaya (*Carica papaya* L.) cv. Rathna. Journal of National Science Foundation Sri lanka. 2008;36(1):41-50.
3. Nakasone HY, Paull RE. Tropical fruits. C.A.B. International; 1998.Allan P, Clark C, Laing M. Grafting papayas (*Carica papaya* L.). Acta Horticulturae (ISHS). 2010;851:254-258.
4. El Moussaoui A, Nijs M, Paul C, Wintjens R, Vincentelli J, et al. Revisiting the enzymes stored in the laticifers of *Carica papaya* in the context of their possible participation in the plant defence mechanism. Cell and Molecular Life Sciences. 2001;58:556-570.
5. Martins DJ. Pollination ecology of papaya (*Carica papaya*) on small-holder farms in Kenya. African Pollinator Initiative. Environment Liaison Centre International; 2009.
6. Imungi JK, Wabule MN. Some chemical characteristics and availability of vitamin A and C from Kenyan varieties of papaya (*Carica papaya* L.). Ecology of Food and Nutrition. 1990;24:115-120.
7. Asudi GO, Ombwara FK, Rimberia FK, Nyende AB, Ateka EM, et al. Morphological diversity of Kenyan papaya germplasm. African Journal of Biotechnology. 2010;9(51):8754-8762.
8. Bhattacharya J, Khuspe SS. *In vitro* and *In vivo* germination of papaya (*Carica papaya* L.) seeds. Scientia Horticulturae. 2001;91:39-49.
9. Panjaitan SB, Aziz MA, Rashid AA, Saleh NM. *In vitro* plantlet regeneration from shoot tip of field-grown Hermaphrodite Papaya (Carica papaya L. Cv. Eksotika). International Journal of Agriculture and Biology. 2007;6:827-832.
10. Allan P, Clark C, Laing M. Grafting papayas (*Carica papaya* L.). Acta Horticulturae. (ISHS). 2010;851:254-258.
11. Rajan S, Markose BL. Propagation of horticultural crops. Horticulture series. 2007;2:16.

12. Saker MM, Bekheet SA, Taha HS, Reda AA. *In vitro* Propagation of Papaya (*Carica papaya* L.) Plant Cell and Tissue Culture Department, National Research Centre, Dokki, Cairo, Egypt; 1999.
13. Cheah LS, Yau KK, Subramaniam S, Lai ET. Cleft grafting and quality improvement of papaya cv Eksotika. Paper presented at seminar on the fruit industry in Malaysia, 7-9th September 1993. Johor Bahru, Malaysia; 1993.
14. Chong ST, Prabhakaran R, Lee HK. An improved technique of propagating 'eksotika' papaya. Acta Horticulturae. (ISHS). 2005;787:273-276.
15. Mohammad AA, Mohammad BH. *In vitro* Micro-grafting of some Iranian grapevine cultivars. Romanian Biotechnological Letters. 2010;15(5):5576-5580.
16. Hartmann HT, Kester DE, Davies FT, Geneve RL. Plant propagation. Principles and practices, seventh ed. Prentice Hall, Upper Saddle River, NJ. 2002:849.
17. Zilka S, Faingersh E, Rotbaum A, Tam Y, Spiegel S, Malca N. *In vitro* production of virus-free pear plants. Acta Horticulturae. 2002;596:477-479.
18. Zimmerman JL. Somatic embryogenesis: A model for early development in higher plants. Plant Cell. 1993;5:1411-1423.
19. Jonard R. Micrografting and its applications to tree improvement. In: Y.P.S. Bajaj (ed.). Biotechnology in agriculture and forestry. Trees I. Springer-Verlag, Berlin. Jonard, R., I. Soedharma, and P. Villemur. 1986;1:31-48.
20. Barros MT, Hipolito FC, Baptista CGM. *In vitro* rooting of Portuguese pear cultivars (*Pyrus communis*) in response to changes in auxin induction and dark period treatments. Acta Horticulturae. 2005;671:631-636.
21. Navarro L, Roistacher CN, Murashige T. Improvement of shoot-tip grafting *In vitro* for virus-free citrus. Journal of American Society of Horticultural Science. 1975;100:471-79.
22. Mumo N N, Rimberia FK, Mamati GE Kihurani AW. *In vitro* regeneration of selected Kenyan papaya (*Carica papaya* L.) lines through shoot tip culture. African journal of Biotechnology. 2013:12(49):6826-6832.
23. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco cultures. Physiology of Plant. 1962;15:473-497.
24. Moore R. A model for graft Compatibility-incompatibility in higher plants. American journal of Botany. 1984;68(6):820-830.
25. Oda M, Maruyama M, Mori G. Water transfer at graft union of tomato plants grafted onto Solanum rootstocks. Journal of Japanese Society of Horticultural Science. 2005;74:458–463.
26. Moore R, Walker DB. Studies on vegetative Compatibility-incompatibility in higher plants. I. A structural study of a compatible autograph in Sedum telephoides (Crassulaceae) Am. J. Bot. 1981;68:820–830.
27. Torii T, Kawazaki M, Okamoto T, Kitani O. Evaluation of Graft-take using a thermal camera. Acta Horticulturae. 1992;319:631–634.
28. Kawaguchi M, Taji A, Backhouse D, Oda M. Anatomy and physiology of graft incompatibility in solanaceous plants. Journal of Horticultural Science and Biotechnology. 2008;83:581-588.
29. Breen PJ, Muraoka T. Seasonal nutrient levels and peach/plum incompatibility. Journal of the American Society for Horticultural Science. 1975;100:339–342.
30. Parkinson M, Jeffree CE, Yeoman MM. Incompatibility in cultured Explant-grafts between members of the Solanaceae. New Phytologist. 1987;107:489–498.

31. Pospisilova J, Solarova AJ Catsky J. Photosynthetic responses to stress during *In vitro* cultivation. Photosynthetica. 1992;26:3-18.
32. Richardson FVM, Saoir SM, Harvey BMR. A study of the graft union in *In vitro* micrografted apple. Plant Growth Regulation. 1996;20:17-23.
33. Pina A, Errea P. A review of new advances in mechanism of graft Compatibility-incompatibility. Scientia Horticulturae. 2005;106:1-11.
34. Rafail ST, Mosleh MS. Factors involved in micropropagation and Shoot-tip grafting of apple (*Malus domestica* borkh.) and pear (pyrus sp. l.). Tropentag, September 14-16, 2010 in Zurich "World Food System – A Contribution from Europe".

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=486&id=32&aid=4250