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RESEARCH NOTE

Jatropha curcas micrografting modifies plant architecture and increases tolerance to abiotic stress: grafting modifies the architecture of *Jatropha curcas*

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Abstract *Jatropha curcas* microshoots excised from well-established multiple shoot cultures of CSMCRI-1 were used as microscions. Different age microscion (6 to18-month-old shoots) and rootstock (15 to 90-day-old) were used for grafting. Among all the different combinations, 18-month-old scions and 60-day-old rootstocks developed grafts with 58.4% success rate. The grafting efficiency was further increased by treating scion and rootstock with 0.05 mg/L BAP (6-benzylaminopurine) and 0.05 mg/L ZN (zeatin). BAP treated scion grafted on 60 days old seedlings established early union. Graft union was dressed with tissue paper wetted with 0.1% Carbemedozo and Macrinite (CM-75) and 400 mg/L amoxicillin-clavulanic acid for infection free graft. Dressing significantly increased the rate of survival (97%) of the grafted plants. 97% microshoots were successfully rooted via grafting. Grafting altered the plant architecture and made them dwarf. The net photosynthetic rate of grafted plants was higher than non-grafted plants. The seed yield of grafted plants was significantly higher than cuttings and seedling.

Keywords Amoxicillin-clavulanic acid · Cuttings · Carbemedozo · Grafting · *Jatropha* · *Curcas* · Microshoot · Photosynthesis

Introduction

Jatropha curcas is well known as biodiesel plant. It is a versatile plant and moderately adapted to different climatic conditions. The use of wasteland for *Jatropha* cultivation removes the unwanted competition for arable land (Divakara et al. 2010). It can be propagated through seedlings; however, cultivation through seedling causes variability in yield due to cross pollination in the plant (Heller 1996). To maintain the consistent traits, vegetative propagation through cutting or by tissue culture is desirable. However, seedlings are found to have better anchoring and tolerance to abiotic stress probably due to taproot system (Lynch 1995). Plants growing in wasteland/arid region are prone to drought and cyclones. Recently, cyclone phailin occurred in October 2013 caused damage to *J. curcas* cutting generated plantation (CSMCRI) at Ganjam, Odisha, India. It is well known that grafted plants subjected to adverse conditions often exhibited better anchoring, greater growth and yield (Rivero et al. 2003). The plant propagated through cuttings are found sensitive to water stress than seedlings because of different root systems (Karlsson and Russell 1990). Mohammed and Vidaver (1991) found that tissue cultured plantlets were more susceptible to water stress as compared to seedlings and concluded that this was not due to physiological differences within the shoots, but was due to differences in root systems. Grafting is considered an environment-friendly operation of significance and sustainable relevance in organic crop management systems (Rivard and Louws 2008).

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Micrografting has been applied in a range of plants to get, successful rooting, higher yield, dwarf plant and resistance against virus and other diseases. Some successful micrografting attempts have been made for fruit trees (Ke et al. 1993; Onay et al. 2004; Richardson et al. 1996; Aleza et al. 2016; Ribeiro et al. 2015), cashew (Thimmappaiah et al. 2002).

In the present study, a novel method has been developed to root microshoots. For the first time a system has been developed of dressing the graft union with fungicide and antibiotic for achieving high grafting success. Also, it was shown by the study that grafting microshoots on seedling of *J. curcas* modified the plant architecture and enhanced the yield.

Materials and methods

The high yielding genotype CSMCRI-1 (Supplementary Fig. 1a) growing in wasteland (shallow soil of 15 cm, rocky and stony) at CSMCRI experimental trial field at Neswad (21°30.49′N; 72°2.19′E), Bhavnagar, Gujarat, India was used in the present study. Nodal explants of CSMCRI-1 were cultured on MS (Murashige and Skoog 1962) medium supplemented with 2.0 mg L^{-1} BAP + 0.2 mg L^{-1} IAA. They were subcultured on MS medium supplemented with 0.5 mg L^{-1} BAP + 1.5 mg L^{-1} IAA for shoot proliferation and elongation, after 4 weeks. Well developed 4–5 cm microshoots of 6, 12, 18, and 24 months were used as scion. The ex vivo rootstocks were raised by germinating presoaked seeds of CSMCRI-1. Sprouted seeds (2 seeds/cup) were transferred into a disposable cup containing sterile sand, wetted with Hoagland solution and maintained at 25 ± 2 °C under cool white florescent light of 88 µmol m^{-2} s^{-1} with 16 h photo-period. The seedlings of different age, 15, 30, 60 and 90-day-old were used for the establishment of graft unions. Scions were cleft grafted on V-shape cut hypocotyl (Supplementary Fig. 1b, c) of 15, 30, 60 and 90-day-old seedlings, held together by wrapping with parafilm and covered with a transparent polyethylene bag. The union success percent in terms of emergence of new leaves was recorded in 4 weeks. Different age scion and 60-day-old rootstock were treated with BAP (0.05 mg/L) and ZN (0.05 mg/L) and held together by parafilm for union establishment. For infection free grafting, dressing was made of 4 × 2 cm tissue paper piece wetted with a solution of 0.1% Carbemedozo and Macrinite (CM-75) + 400 mg/L amoxicillin-clavulanic acid and the same was wrapped around the union and thereafter tied tightly with parafilm. Grafted plants were placed in 80% humidity and 25–26 °C temperature to heal the graft union. The union success percent was recorded in 2 weeks and the union formation was confirmed by removing wrapped parafilm. Net photosynthetic rate, concentration of intercellular CO_2 and transpiration rate of leaves was noted of 2-month-old plant in greenhouse with a portable photosynthesis system (LI-6400XT, Li-Cor Inc, Nebraska, USA), (300 µmol photons m^{-2} s^{-1}). Height of 4-month-old grafted plants was recorded. The yield of 1-year-old grafted (TC-G) plants was compared with the same age stem cuttings and seedlings. The values of data are mean ± standard error of replicates. Each treatment comprised 10 replicates of scions and the experiments were analysed by completely randomized design. Data were subjected to analysis of variance (ANOVA), analysed by two factors CRD analysis. Height and yield were recorded as the mean value of five replicates plants. All statistical analysis was performed by SPSS version 7.5 at the 5% level.

Results and discussion

The graft union success rate was dependent on the rootstock and scion age. On comparing success rate of grafts carried out with 15, 30, 60 and 90-day-old rootstock, we observed that 30 to 60-day-old rootstock success rate was higher. The best graft union success was achieved with 18-month-old microscion as compared to 6 and 12-month-old (Fig. 1). However, some of grafts died due to fungal and bacterial infection at the graft union (Supplementary Fig. 1d). The success rate was higher in BAP and ZN treated grafts as compared to untreated grafts (Figs. 1, 2). The treatment with BAP resulted in a significantly higher survival rate (75.4%) with no callusing at the graft union (Fig. 2), usually formed due to PGRs. This might be due to the use of low concentration of cytokinin in the present study. Callus formation was found to be essential in tomato for successful graft union (Goldschmidt 2014). Fungal and bacterial infection at the graft union caused 22–36% death of grafted plants (data not shown). This may be due to the different growing conditions of the scion (in vitro sterile) and rootstock (ex vitro in soil). To control infection, graft union was dressed with a solution containing 0.1% CM-75 and 400 mg/L amoxicillin-clavulanic acid (Supplementary Fig. 1e). Treatment of 0.1% CM-75 + 400 mg/L amoxicillin-clavulanic acid controlled infection up to 100 percent. BAP and dressing treatment have a significant effect on the success rate of establishment as well as on the extended survival rate of the grafted plant. CM-75 is reported to be a potent fungicide in controlling collar rot disease in *J. curcas* caused by *Macrophomina phaseolina* (Singh et al. unpublished data). Chong et al. (2008) reported an initial grafting success of 80%, although this was reduced later due to infection of soft-rot fungi. Hence, graft union needs to be infection free for higher success rate of establishment. The highest survival of 97% was achieved by dressing of graft union (Fig. 2). Plants took 2–3 weeks to completely heal the graft union

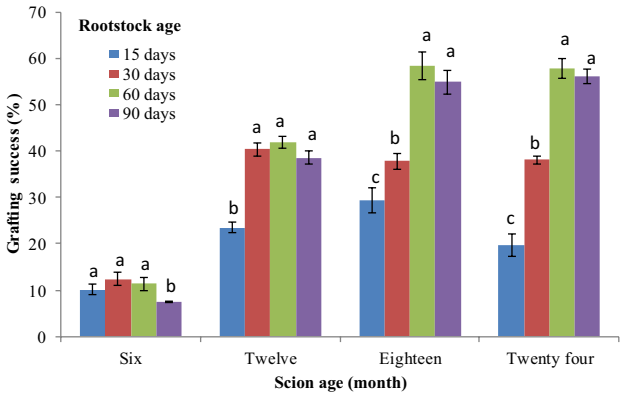

Fig. 1 Effect of scion age and rootstock age on the graft union success of *Jatropha curcas*. *Values* represent means ± SE of 10 replicates; *different letters* within the treatments are significantly different at 0.05 probability level

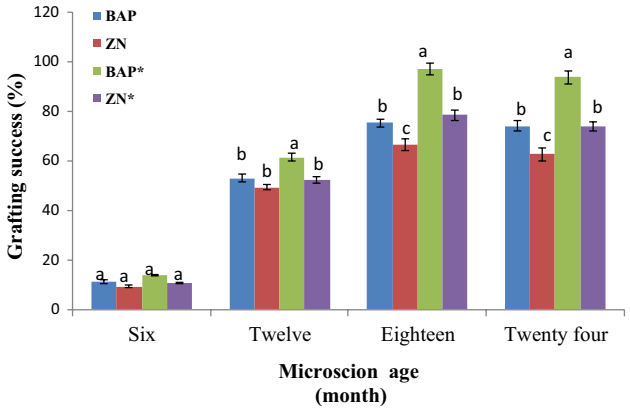

Fig. 2 Effect of hormone and dressing on grafting success of *Jatropha curcas*. BAP 6-benzylaminopurine, ZN zeatin, *Dressing of 0.1 % CM-75 + 400 mg/L amoxicillin-clavulanic acid on graft union, *Values* represent means ± SE of 10 replicates, *Different letters* within the treatments are significantly different at 0.05 probability level

(Supplementary Fig. 1f) and 98 % plants successfully established in greenhouse and in pots (Supplementary Fig. 1g). Microshoots rooted by grafting method took 4 weeks to reach nursery and in vitro rooting method takes 10 weeks to reach greenhouse (Singh et al. 2010) and 5 weeks more to reach the nursery. Hence, grafting method is better than in vitro rooting method in terms of high rooting percentage, short time taken for rooting and better anchoring of the plant with soil due to taproot system. The height of grafted plants (TC-G) were significantly shorter compared to height of non-grafted plants [stem cuttings (C) and seedling plants (Supplementary Fig. 2)]. These results revealed that grafting induced dwarfing in the plants. The results obtained in the present study indicated that grafting had modified the architecture of the plants. Dwarfing in apple by grafting has been reported (Hooijdonk et al. 2010). Lower endogenous concentration of cytokinins in the xylem sap of the scion might have contributed to scion dwarfing in apple (Kamboj et al. 1999). Bhogale et al. (2014) found microRNA 156 a potential graft-transmissible signal that affected *Solanum tuberosum* architecture and tuberization. The involvement of miRNAs in grafting has been demonstrated by Tzarfati et al. (2013).

Yield performance of grafted plants (Supplementary Fig. 1h) was better than non-grafted (cuttings and seedlings) plants (Supplementary Fig. 3). Grafting has been made in many plants, aiming to alter root traits that can develop more stress tolerant and high yielding crop (Paez-Garcia et al. 2015). The enhanced yield of the grafted plants of *J. curcas* might be due to taproot system in contrast to a fibrous root system of cuttings. Taproots have better anchoring and stress tolerance (Paez-Garcia et al. 2015). In grafting, there are chances of chloroplast genomes transfer and this way it opens the possibilities of plant breeding that are obstinate to plastid genome transformation (Stegemann et al. 2012). In the present study, grafting moderately enhanced net photosynthesis rates, however, the concentration of intercellular CO_2 and transpiration rate was not significantly different among the plants (Supplementary Fig. 4). Grafting techniques also provide possible path for horizontal gene transfer (Stegemann and Ralph 2009). Grafted *J. curcas* plants were dwarf and high yielding compared to non-grafted plants.

In the present study, a new infection free micrografting method has been developed. The optimum grafting union success was achieved by BAP and dressing treatment. This method will be helpful in generating planting material with better traits (dwarf, high yielding and taproot) for large scale propagation in stress condition. This study also opens the door for further studies like hetrografting to develop new traits.

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