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Research Article
Genotypic Variation Affects the Efficiency of the Genetic Transformation Process in *Balanites aegyptiaca*

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Abstract

Background and Objective: *Balanites aegyptiaca*, belonging to family Zygophyllaceae, is multipurpose tree considered highly drought-tolerant but salt-sensitive and cultivated in several arid and tropical regions in North and west Africa and West Asia. The plant parts contain several secondary metabolites and there is a high oil content in the kernels already used in biodiesel production. This study was aimed at investigating effect of the genotypic variation in *B. aegyptiaca* plants (collected from different geographical locations, Riyadh, Saudi Arabia and Halayeb, Egypt) on the efficiency of the genetic transformation process for both *ERD10* and *nptII* genes. **Materials and Methods:** Two *B. aegyptiaca* genotypes collected from two different geographical regions with different ecological conditions (Halayeb, Egypt and Riyadh, Saudi Arabia) were used in this study. The genetic transformation was performed using *Agrobacterium tumefaciens*s train GV3101 harboring the binary vector pBinAR containing *ERD10* (Early Responsive to Dehydration 10) and *nptII* genes, to produce salt-tolerant *B. aegyptiaca* plants. **Results:** The results showed that each genotype collected from the different geographical locations after transformation exhibited significant differences in the number of leaves per regenerated explant and shoot length, where by nodal explants from Halayeb, Egypt exhibited the highest values. **Conclusion:** Current findings emphasize the impact of the genotypic variation (geographical location) of the used plant on the efficiency of the genetic transformation process.

Key words: *Agrobacterium tumefaciens*, genetic transformation, biodiesel production, *Balanites aegyptiaca*, drought-tolerant, genotypic variation, salt-tolerant

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Balanites aegyptiaca (L.) Del, commonly called the desert date, is a xerophytic multipurpose tree species belonging to family Zygophyllaceae and is considered a highly drought-tolerant plant species found naturally in arid and semi-arid regions in north and west Africa, west Asia and the Arabian Peninsula[1]. Chapagain *et al.*[2] reported that oil content represents approximately 47% of the kernel and this oil is effectively analyzed for biodiesel production after esterification. Several parts of *B. aegyptiaca* (seeds, leaves, roots and fruits) show biological activities, such as antibacterial, anticancer and antifungal activities for medical treatment and oral contraceptive production, owing to the presence of various secondary metabolites, apart from saponins, which are successfully used in detergent production, including foaming agents[2-4].

Salinity has a negative impact on plant productivity and food production and salt stress affects 800 million ha of agricultural land worldwide[5]. Despite the high capacity of *B. aegyptiaca* as a drought-tolerant plant, *B. aegyptiaca* seedlings show high sensitivity to salt stress at low levels (12 dS m^{-1}). However, salt stress at high levels (24 dS m^{-1}) also showed harmful effects on seedling growth[6]. Furthermore, salt stress negatively affects the biomass and growth of three different *B. aegyptiaca* showing significantly differences in responses to salt stress[7]. Therefore, there is a demand for improving salt tolerance in *B. aegyptiaca* to cultivates it in arid and semi-arid regions with various soil types. Kovacs *et al.*[8] reported that under abiotic stress, such as salt and drought stress, accumulation of early responses to dehydration proteins (ERD10), which are members of the Dehydrin family, occurs. The chaperone activity of *ERD10* might protect proteins by preventing the inactivation and heat-induced aggregation of various proteins under stress[9].
Several constraints affect the propagation of woody plant species. Nevertheless, numerous classical techniques were used for propagating *B. aegyptiaca* using roots, suckers, seeds and cuttings; however, these methods showed limitations for the mass propagation of this plant species, owing to slow growth and low germination rate of seeds[10]. The use of biotechnological techniques, such as *in vitro* propagation, for trees has shown considerable potential for large-scale multiplication, apart from the improvement and conservation of elite clones[11]. Several efforts were made for the *in vitro* propagation of *B. aegyptiaca* using nodal explants with axillary buds, nodal and cotyledonary parts[10,12,13] somatic embryos through roots segments[14] callus induction were obtained through roots and cotyledon explants and apical buds[15]. Khamis *et al.*[13] reported the first reporton the transformation of *B. aegyptiaca* using three different *Agrobacterium tumefaciens* strains (EHA105, GV3101 and LBA4404), harboring the pCAMBIA2301 plasmid containing the *nptII* marker and *gus* reporter genes, where strain GV3101 showed the highest transformation efficiency. In addition, they transferred *ERD10* gene into *B. aegyptiaca* producing a salt tolerant transformed plant. However, *B. aegyptiaca* plants collected from different locations revealed genetic variation with a high percentage of polymorphism[16,17]. Transformation efficiency to introduce *ERD10* gene into *B. aegyptiaca* plants of different genotypes has not been investigated. This study was aimed at investigating effect of the genotypic variation in *B. aegyptiaca* plants (collected from different geographical locations, Halayeb, Egypt and Riyadh, Saudi Arabia) on the efficiency of the genetic transformation process for both *ERD10* and *nptII* genes using *Agrobacterium tumefaciens* containing the pBinAR vector harboring *ERD10*.

MATERIALS AND METHODS

Balanites aegyptiaca need 6 months for regeneration and acclimatization. So, this experiment was conducted between June and December, 2017.

Plant material and culture conditions: Seeds of *B. aegyptiaca* were collected from two different geographical locations, Saudi Arabia (Riyadh) and Egypt (Halayeb). The seeds were mechanically released from the fruits. The seeds were sterilized with sodium hypochlorite (9.4% active chlorine) for 40 min and washed four times with sterilized water. For germination, the seeds were placed on Murashige and Skoog (MS) medium supplemented with 3% (w/v) sucrose and 0.4% Phytagel and pH was adjusted to 5.7 for 4 weeks[18] and incubated in a growth chamber at 24°C with a 16:8 h light:dark photoperiod. After germination, shoot segments containing single node were excised for use as nodal explants. Preparation of *Agrobacterium tumefaciens* and explants for transformation.

The binary vector pBinAR harboring *ERD10*[13] was transformed by the freeze/thaw shock method of transformation[19] into *A. tumefaciens* strain GV3101. The cells were spread on 2× yeast extract-tryptone (2-YT) plates supplemented with rifampicin (50 mg L^{-1}) and kanamycin (50 mg L^{-1}). On the second day of incubation, the transformed colonies of strain GV3101 were subjected to plasmid isolation and the presence of *ERD10* (800 bp) was detected by PCR as described by Khamis *et al.*[13].

Preparation of nodal explants for transformation and regeneration conditions: At the 4th week of germination, the nodal explants were excised from aseptic seedlings of *B. aegyptiaca* from Halayeb, Egypt (E) and Riyadh, Saudi Arabia (S). Preparation of nodal explants for transformation was conducted according to Khamis *et al.*[13]. For acclimatization, healthy plantlets with shoots and roots (Two transformed plantlets from Riyadh, Saudi Arabia and three from Halayeb, Egypt) were transferred under greenhouse conditions to pots filled with soil (natural clay and peat, 2:1) and covered with plastic bags to maintain humidity and watered every two days with half-strength MS medium for two weeks. Then, the plastic bags were removed to allow the plantlets to adapt for another two weeks.

Molecular analysis: Polymerase Chain Reaction (PCR) was conducted according to Khamis *et al.*[13] for detecting the presence of *ERD10* and *nptii* in the transformed plants. Leaves were taken from regenerated, transformed plants and DNA was extracted by the cetyltrimethylammonium bromide (CTAB) method. The following primers were used: *nptii*5'ATGGCTAAAATGAGAATA3' as the forward primer and *nptii* 5'CTAAAACAATTCATCCAG3' as the reverse primer. The amplification of the 800 bp PCR fragment of *ERD10* through PCR was performed using the following primers: Forward primer, 5'GGTACCATGGCTGAAGAGTACAAG3' and reverse primer, 5'GGATCCTCATCCTTCTAAATCATCGG3'.

Statistical analysis: Approximately, 150 nodal explants were used in the transformation experiment for both genotypes from both locations in Saudi Arabia and Egypt. Different analytical measurements for the biological samples were performed in duplicate and the results were expressed as Means±SE. Independent t-tests were performed to determine whether the differences between the two groups were statistically significant ($p<0.05$) using SPSS 17 for Windows.

RESULTS

Sterilized seeds (150) of each *B. aegyptiaca* genotype were placed on MS medium without PGR; after 4 weeks of germination, the seeds from Egypt (Halayeb) showed a higher germination percentage than those from Saudi Arabia (Riyadh). The transformed nodal explants from each location were responsive for shoot formation (Fig. 1a-d), when placed on MS medium supplemented with 8.8 μM BA and kanamycin (100 mg L^{-1}) as the selected marker. The transformed nodal explants from Riyadh exhibited a higher percentage of the number of explants produced shoots (37%) than those from Halayeb (33%).

The responses of the transformed plantlets from each location were significantly different (Table 1) after 4 weeks of inoculation on MS medium supplemented with 8.8 μM BA, 1.3 μM NAA and kanamycin (200 mg L^{-1}). The transformed plantlets from Halayeb showed the highest number of shoots per regenerated explants (3.4±0.4) and highest shoot length (1.6±0.8) and they were significantly higher than those from Riyadh (0.96±0.16 and 0.44±0.04) for the number of shoots per regenerated explants and shoot length, respectively (Table 1). Root induction was observed when the transformed plantlets were moved to MS medium supplemented with 1.2 μM NAA and kanamycin (200 mg L^{-1}). Only three plants from Halayeb and two plants from Riyadh produced roots and survived at a high concentration of kanamycin (200 mg L^{-1}) and then, these plants from each location were transferred to the soil for acclimatization.

(a) (b) (c) (d)

Fig. 1(a-d): Multiple shoot formation in *B. aegyptiaca* from Halayeb, Egypt using nodal explants after transformation with *A. tumefaciens*, (a) Nodal explant on the first day, (b) Multiple shoot induction on MS+8.8 μM BA after 3 weeks, (c) Elongation and proliferation of multiple shoots on MS+8.8 μM BA+1.3 μM NAA after 8 weeks and (D) *In vitro* rooted plantlet on MS+1.2 μM NAA

Fig. 2(a-b): PCR products of (a) *ERD10* (800 bp) and (b) *nptII* (750 bp)

M: One kbp DNA ladder, +: Binary vector pBinAR containing *ERD10* and *nptII* and -: Leaves excised from non-transformed *B. aegyptiaca* plants. E1, E2, E3: Leaves of *B. aegyptiaca* from Egypt, S1, S2: Leaves of *B. aegyptiaca* from Saudi Arabia. Both *B. aegyptiaca* plant genotypes were transformed using. *A. tumefaciens* strain GV3101 harboring the binary vector pBinAR containing *ERD10* and *nptII*

Table 1: Frequencies of shoot regeneration for the two *B. aegyptiaca* genotypes after transformation with *A. tumefaciens*

Location	No. of explants produced shoots (%)	No. of shoots per regenerated explant	Shoot length (cm)
Halayeb, Egypt (E)	33	3.4±0.4*	1.6±0.8
Riyadh, Saudi Arabia (S)	37	0.96±0.16*	0.44±0.04

Values represent Means±SE, Means followed by *Within a column differed significantly after Tukey's test ($p<0.05$)

Leaves of the three transformed plants from Halayeb, Egypt (E), two from Riyadh, Saudi Arabia (S) and non-transformed (-) *B. aegyptiaca* plants were subjected to PCR analysis. The presence of *ERD10* was detected in the transformed plants (E1, E2 and E3) from Halayeb, Egypt (Fig. 1a). Furthermore, the presence of *nptII* was detected in the transformed plants (E1 and E2) from Halayeb, Egypt (Fig. 2b). However, both the transformed plants from Riyadh, Saudi Arabia (S) and non-transformed plants did not show the respective fragments (Fig. 2a, b).

DISCUSSION

In this study, *B. aegyptiaca* seeds collected from two different geographic locations, Saudi Arabia (Riyadh) and Egypt (Halayeb) were studied to investigate the responses of the two *B. aegyptiaca* genotypes to genetic transformation. The transformation process was conducted using *A. tumefaciens* strain GV3101 harboring the binary vector pBinAR containing *ERD10* and *nptII* to increase the salt tolerance in *B. aegyptiaca* through axillary shoot formation from nodal explants. The BA was effective in inducing shoot formation in the two genotypes. The obtained results are in agreement with those reported in the previous studies on *B. aegyptiaca*[10, 12,20]. Balanites sources collected from different locations showed genetic variation and revealed high percentage of polymorphism[17]. The relation between genetic variation in *B. aegyptiaca* and geographical locations was investigated by Chamberlain[16], showing that there is a high variability in the response to the expression of peroxidase isozyme between plants from different locations. The results showed that after transformation, the seeds collected from the different geographical locations exhibited no significant differences in the number of explants produced shoots between the two locations. Nevertheless, significant differences between the plants collected from the two locations in the number of shoots per regenerated explant and shoot length and the highest values were recorded in the plants from Halayeb, Egypt. This variation might be related to the genotypic variation between the seeds, which were collected from two different locations. However, *B. aegyptiaca* is a woody plant species considered partially auto-compatible and exhibited a cross-pollination rate of approximately 37%, which could be related to wind and insects[12,21]. Moreover, the plant species. *Aegyptiaca* is classified into varieties (*ferox, pallida*, *quarrei* and *tomentosa*), which are highly variable in their visible characteristics related to attributes such as distribution, flowers, fruits and preferred soil type[22]. High intraspecific genetic diversity was reported for the woody species with outcrossing breeding systems and they are distributed in wide geographical ranges[23]. Only three transformed plants from Halayeb and two from Riyadh produced roots and survived. The effects of genotype on multiplication rate

were investigated in *Juglansregia*, where by certain clones showed a high rooting percentage (95%) and few had a low percentage (5%) [24]. The presence of both *ERD10* and *nptII* was confirmed via PCR analysis of leaves excised from the plants from Halayeb; however, the transformed plants from Riyadh and non-transformed plants did not exhibit the respective fragments. Variation in genetic transformation among genotypes or varieties within the same plant species was reported for different wheat cultivars, whereby Gemmiza 10 and Gemmiza 9 exhibited 80 and 50% positive PCR results for *nptII*, respectively[25]. Liu *et al*. [26] reported that, there were significant variations in transformation efficiency among five banana (*Musa* spp.) varieties, wherein the highest transformation efficiency was recorded for the Gongjiao variety (9.81 ± 0.29^{a}) and the Baxi variety exhibited the lowest transformation efficiency (1.16 ± 0.15^{c}). Chateau *et al*.[27] showed that several *Arabidopsis* ecotypes showed differences in their susceptibility to *Agrobacterium*-mediated transformation and their types of responses to pre-cultivation. Under abiotic stress, such as cold and salt stress, leaves of *Brassica napus* L. exhibit high dehydrin *ERD10* expression as a mechanism to overcome salt stress[28]. Moreover, *ERD10* plays a substantial role in seed development and increases the capability of plants to cope with stress; furthermore, the dehydrin ERD10 is important for tolerance to dehydration[9,29].

CONCLUSION

The obtained findings emphasize the impact of the genotypic variation of the used plant on the efficiency of the genetic transformation process. However, further investigations are required to study the expression of the transformed genes and the ability of the transformed plant to survive under different salinity levels. This study may help in the production of salt-tolerant plants which can be cultivated in arid and semi-arid lands and may help in some biotechnological industries such as the biodiesel production.

SIGNIFICANCE STATEMENT

This study may help in the production of salt-tolerant *B. aegyptiaca* plants which can be cultivated in arid and semi-arid lands which may help in some biotechnological industries such as the biodiesel production.

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