PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 9(sp3): 16–24
https://doi.org/10.14719/pst.1764

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RESEARCH ARTICLE

Establishment of *in planta* transformation protocol of tomato (*Solanum lycopersicum* L.) through antiporter gene for improved salinity tolerance

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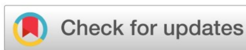
ARTICLE HISTORY

Received: 19 March 2022
Accepted: 23 June 2022

Available online
Version 1.0 : 06 September 2022



Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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Datta A, Ferdous M E M, Islam A. Establishment of *in planta* transformation protocol of tomato (*Solanum lycopersicum* L.) through antiporter gene for improved salinity tolerance. Plant Science Today. 2022;9(sp3): 16–24. https://doi.org/10.14719/pst.1764

Abstract

Tomato stands as the world's third most consumed vegetable, but its production has been suffering due to climate vulnerability, notably for saline sensitivity. Despite its economic importance, developing salinity tolerant tomato has not been prioritized lately. Current study was aimed to establish a simple and efficient *Agrobacterium*-mediated *in planta* transformation protocol to transform Na^{+}/H^{+} antiporter gene into 5 Bangladeshi tomato varieties, namely BARI tomato 2, BARI tomato 3, BINA tomato 2, BINA tomato 3 and Bahar, to improve their salt tolerance, through optimization of crucial transformation factors like optical density, infection time, co-cultivation period etc. Two vectors were constructed by cloning Na^{+}/H^{+} antiporter gene from *Arabidopsis* (*pK7WG2_AtNHX1_1.6*) and Rice (*pK7WG2_OsNHX1_1.6*) individually to gateway vector pENTR/D-TOPO and electroporated to *Agrobacterium* while another vector *pBI121* was used as control. Non-pricked seeds were found optimum for achieving more than 90% efficiency for GUS expression and germination percentages under conditions of OD_{600} 1.1-1.4 with 30 min of infection time followed by 24 hrs co-cultivation period during transformation using the 3 vectors. Transformed plantlets were screened through resistance to Kanamycin 50 mg/l in germination medium while Cefotaxime 100 mg/l was applied to prevent *Agrobacterium* overgrowth during co-cultivation. Tolerance of 100 mM NaCl for 14 days has been observed in putative transformants in Leaf Disc Bioassay. No significant morphological changes were observed during the acclimatization of putatively transformed plantlets. This established protocol is novel and can efficiently produce genotype-independent transgenic tomato plants obviating intervening tissue culture. Hence, this study provides scope for climate-resilient crop improvement to ensure nutritional security.

Keywords

Salinity, Tomato, *Agrobacterium*, *In planta* transformation, Antiporter, Leaf disk assay

Introduction

Tomato (*Solanum lycopercicum* L.) ranks as 'favorite' among vegetables considering its increasing global consumption which presently covers more than 19% of all vegetable intakes worldwide. It is a source of essential nutrients with ample antioxidants to provide required nutrients in the everyday diet for the malnourished underprivileged people (1). Ensuring nutritional security has been a challenge to many countries including Bangladesh.

Twenty-five % of the current population faces limited access to nutritious food which demands much enhanced vegetable production (2). Tomato has been ranked third in global vegetable production, but climate vulnerability, specially salinity in cultivable land and water play a major role in achieving this as more than 1/5 of cultivation takes place at the coastal area (3). Regarding this fact, the most devastating contributor is the increasing salinization of cultivable land and water, which has been estimated to affect more than 50% of current farming land by the end of 2050 (4, 5). The saline condition can be accounted for lower production and failure to meet national demand (6). In such a situation, the salt-tolerant tomato variety can be a key tool to reinforce its cultivation in the saline-prone areas of Bangladesh; hence can contribute to sustaining nutritional security.

For the last two decades, great efforts have been made to develop salinity-tolerant crops. Although there are comparatively salt-tolerant relatives of tomato exists, still conventional breeding remain unsuccessful in achieving the desired goal (7). *In vitro* regeneration of tomato, followed by transformation with salt tolerant trait has been tried in many countries, including Bangladesh, to overcome this barrier.

In tomato transformation, cotyledons have been used extensively for *in vitro* regeneration and transgenic generation. However, *in vitro* transformation has certain limitations, i.e., low transformation efficiency, dependency on tissue culture protocol, recalcitrancy of some cultivars, lack of reproducibility, shoot necrosis, problematic root induction and subsequent transplantation, labor-intensive method, time-consuming process etc. (8, 9). Despite optimal conditions provided for regeneration and transformation, incidents like somaclonal variation, morphological deformities, chromosomal aberration, anomalies in plant reproductive characteristics etc. have been reported for tissue culture dependent transformation studies (10). Alternatively, *in planta* transformation offers several benefits as it can avoid somaclonal variation and there is no report of morphological change, chromosomal alteration and loss of plant reproductivity (10). Previously, *in planta* transformation methods have been reported for tomato using floral dip method, whole fruit injection method, smearing *Agrobacterium* suspension on floral buds but efficiency of seeds as explant in *in planta* transformation is yet to be explored (11, 12).

In this backdrop, we intended to establish an *Agrobacterium tumefaciens*-mediated transformation protocol for 5 Bangladeshi popular tomato varieties, which can overcome the inadequacies of the available approaches of plant transformation as well as efficient, reproducible, genotype and culture-independent. Moreover, in this study instead of using a reporter marker gene, we used salt-tolerant vacuolar antiporters gene like *AtNHX1* cloned from *Arabidopsis thaliana* and *OsNHX1* cloned from *Oryza sativa* L., which has already proved its efficiency in enhancing salt tolerance in tomato (13). Therefore, this study was designed to transform these 2 genes individually into 5 locally grown farmer popular cultivars to achieve improved salt tolerance.

The observations presented here could allow potential genetic modification of this important crop avoiding the disadvantages of the tissue culture-dependent transgenic crop development and help in understanding the effect of these antiporter genes in improving salt tolerance and facilitating the future goal of producing transgenic salt-tolerant cultivars of tomato. There is no research article published on *in planta* transformation protocol on Bangladeshi BARI tomato varieties using mature seeds as explants. However, the same protocol has been used in transformation of BINA varieties (BINA Tomato 2, BINA Tomato 3 and Bahar) using a salinity tolerant antiporter gene *AtNHX1* (14). This study can make way for climate-resilient agriculture utilizing more varieties in future.

Materials and Methods

Plant material collection

Seeds of 5 varieties of tomato were used for transformation study. Among these 5 varieties, 2 varieties were collected from Bangladesh Agricultural Research Institute (BARI) named as BARI tomato 2 (BR2), BARI tomato 3 (BR3) and the remaining 3 varieties were collected from Bangladesh Institute of Nuclear Agriculture (BINA) named as BINA tomato 2 (BN2), BINA tomato 3 (BN3) and Bahar (Bh).

Seed sterilization

Seed sterilization procedure was carried out as described (15). First, 70% ethanol was used for surface sterilization, followed by washing with 20 ml of 30 % Clorox with 2 drops of Tween-20. Seeds were then shaken by rotatory shaker (Model: WIS-20, Korea) for 5 min. After that, the seeds were rinsed well with autoclaved distilled water 3 times to remove any trace of sterilant and finally kept in the rotatory shaker at 28 °C with 120 rpm for overnight to remove the gelatinous layer around the seeds.

Agrobacterium strain and plasmid vectors

For transformation studies, *Agrobacterium tumefaciens* strain LBA4404 harboring 3 individual plasmids constructs, *pBI121* (Fig. 1A), *pK7WG2_OsNHX1_1.6* (collected from Department of Biochemistry and Molecular Biology, University of Dhaka) (Fig. 1B) and *pK7WG2_AtNHX1_1.6* (Fig. 1C) were used (16).

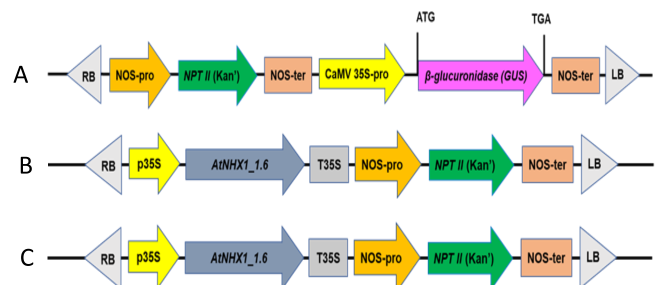


Fig. 1. Schematic diagram of Vectors. **A.** the T-DNA region of the binary *pBI121*, **B.** Constructed vector *pK7WG2_OsNHX1_1.6*, **C.** Constructed vector *pK7WG2_AtNHX1_1.6*.

Media used in different phases of the study

The media compositions and preparations of all types of stock solutions have been mentioned earlier (17). The list

of media used in this experiment is further presented in Table 1).

Table 1. List of media used in this experiment

Media	Used for	Components	Amount
YEP	*Agrobacterium* culture	Yeast extract	10 g/l
		Peptone	10 g/l
		Sodium Chloride	5 g/l
		Stock concentration	**Amount**
MS	Germination, Co-cultivation	Macronutrients (10x)	100 ml/l
		Micronutrients (100x)	10 ml/l
		Fe- EDTA (100x)	10 ml/l
		Organic nutrients (100x)	10 ml/l
		Sucrose	30 g/l
		Myo-inositol	0.1 g/l
		Plant growth hormones	**Amount**
MS + hormone	Regeneration	BAP (6-Benzylaminopurine)	2 mg/l
		IAA (Indole-3 acetic acid)	0.1 mg/l
MS + antibiotic	Selection of transformed plants	Kanamycin	50 mg/l

Determination of baseline saline tolerance level of tomato seedlings

Germination occurred at 25±2 °C with 16 hrs photoperiod and the time required for seed germination and seedling development was recorded. Seed germination referred to the initial appearance of radicle and its length was measured at approximately 2 mm. Germination rate was counted every day (up to the 20th day). Germination efficiency was determined by using the following formula:

$$\text{Germination Efficiency} = \frac{\text{The number of seeds germinated}}{\text{Total number of seeds inoculated}} \times 100$$

This formula was also followed to calculate germination efficiency of putative transformants.

The effect of salinity during germination of tomato seeds was investigated to determine basal salt sensitivity level, ranging from 0-100 mM (0-10 dS/m) was chosen for the study. MS media was prepared with different amount (0 mM, 5 mM, 10 mM, 20 mM, 50 mM and 100 mM) of NaCl in each 100 ml of media. Tomato seeds were subjected in these specified media to test saline sensitivity for seven days and data was recorded.

Antibiotic sensitivity test

Determination of the lethal dose concentration of antibiotics was a critical step in transformation process. Therefore, 10 pieces of cotyledonary leaves was placed to regeneration media containing cefotaxime (0, 50, 100, 150 and 200 mg/l) and kanamycin (0, 7.5, 12.5, 25, 50, 100, 150 and 200 mg/l) for 30 days, to detect the resistance response of tomato.

Procedure of in planta transformation of whole seed explants

In planta is an indirect transformation technique that is mediated by *Agrobacterium tumefaciens* and tissue culture -independent. Several factors were optimized such as, *Agrobacterium* culture density, infection time, co-cultivation period. *Agrobacterium* cells were inoculated in the YEP liquid medium with respective antibiotics and cultured overnight at 28 °C with 180 rpm. Seeds were surface-sterilized before transformation. *Agrobacterium* cells were harvested at different cell densities so that seeds can be immersed in culture for 15-30 min of infection time. The seeds were gently pricked 0-3 times using a sterile needle and immediately transferred into the *Agrobacterium* culture (Fig. 2). The seeds were further transferred to petri

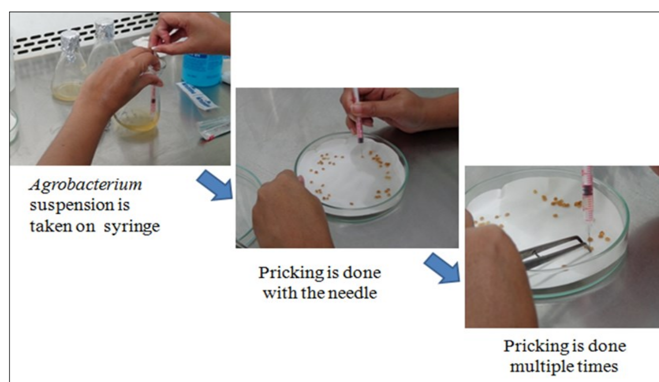


Fig. 2. Process of pricking on whole seed of tomato.

dishes for co-cultivation in the dark at 25 °C for 24-72 hrs. The co-cultivated seeds have been rinsed three times in autoclaved distilled water supplemented with 100 mg/l of cefotaxime to remove excess *Agrobacterium* over the seeds. The seeds were then placed to germination media containing 50 mg/l of Kanamycin. Germination % of control and putative transformed seeds were recorded. In each experiment, 50 seeds were infected, and the experiments were repeated at least 3 times. One month old putative transgenic plantlets were transferred to pots containing autoclaved soil moistened with liquid MS medium or distilled water. The plantlets could grow under growth room conditions for at least 45-60 days before they were transferred to the net house. The plants were allowed to mature and produced seeds for the regeneration of T_1 plants.

GUS histochemical analysis for stable gene expression

This method was carried out to analyze the stable GUS gene expression in tissue segments from the putative transformed seedlings according to the described procedure (17). Tissue segments were immersed in fixation solution in sterile eppendorf tubes and incubated overnight. Then, the tissues were washed at least 3 times with 50 mM phosphate buffer, pH 7.0. After that, the X-Gluc solution was added and incubated at 37 °C overnight to develop the blue color. Next, 70% ethanol was added and again incubated at 37 °C for 48 hrs before observing under stereomicroscope (AmScope, USA).

Leaf disc assay

From control and transformed plants, leaf disks (~2 cm

diameter) were excised and floated on 10 ml of NaCl solution (5 mM, 10 mM, 20 mM, 50 mM and 100 mM) for 14 days. For experimental control 0 mM NaCl (autoclaved distilled water) was maintained at same timeframe. The same were kept under the standard photoperiod at 25 °C. The effect of salt treatment on leaf disks was observed by monitoring phenotypic changes, as browning, bleaching and freshness of leaves (18-20).

Results

Salinity tolerance of tomato seeds

Mature seeds from the 5 varieties have been tested for their salinity tolerance level ranging from 0 mM to 100 mM. All the varieties have been observed to show almost similar response in saline condition. Germination efficiency dropped from 86% to 1.6% when salinity increased from 0 mM to 100 mM respectively (Table 2, here data from BR3 has been presented).

Table 2. Effect of salinity on tomato seed germination

NaCl concentration in germination media (mM)	No. of inoculated seed	Germination of seeds (%)	Mean no. of germinated seeds ± SD
0	20	86.7	17.3±0.57
5	20	76.7	15.3±0.57
10	20	60	12.0±1.0
20	20	46.6	9.3±0.57
50	20	33.3	6.67±1.15
100	20	1.6	0.33±0.57

Mean value is from 3 replicates of BR3, data was taken 14 days after inoculation.

Antibiotics tolerance test for selection of transformed explants

The cotyledonary leaves explants could regenerate in presence of cefotaxime upto 100 mg/l, while necrosis of explants was observed at 200 mg/l (Table 3). Regarding kanamycin tolerance test, the explants became albino at 7.5 mg/l, while death of explants was observed at 50 mg/l (Table 3). Therefore, 100 mg/l cefotaxime and 50 mg/l kanamycin were used in media as it was found to be the most favorable for morphogenesis and selection of transformants (Fig. 3).

Table 3. Effect of antibiotics on the regeneration of tomato cotyledonary leaf

Antibiotic concentration (mg/l)		Regeneration (%)	Survival (%)	Visual Appearance
	0	80	90	Green
	50	65	80	Green
Cefotaxime	100	75	85	Green
	150	45	50	Albino
	200	35	40	Necrosis
	0	70	90	Green
	7.5	15	0.15	Albino
Kanamycin	12.5	08	0.08	Albino
	25	02	0.02	Brown
	50	0	0	Necrosis

Ten cotyledons were subjected in each trial. Values were obtained from three independent trials. These data were collected after 30 days of inoculation and shoot formation rate was recorded after 45 days of inoculation.

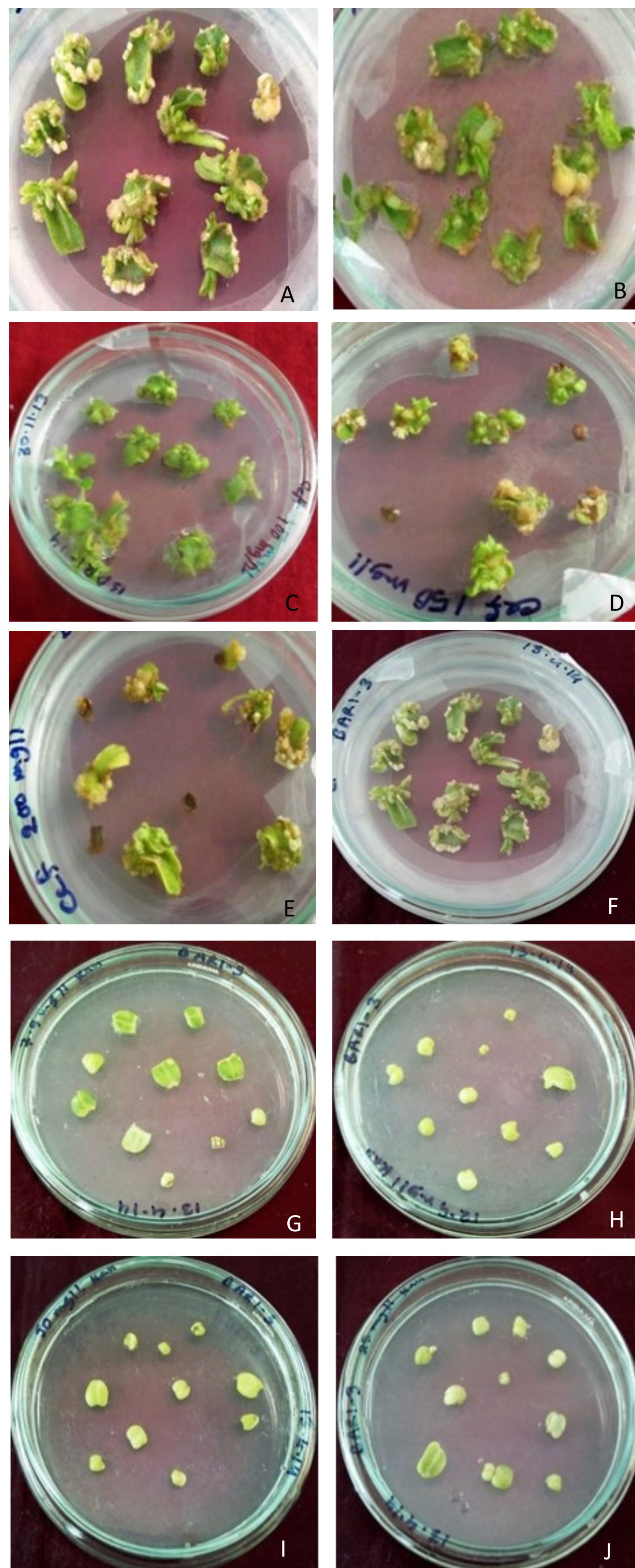


Fig. 3. Effect of various concentrations of cefotaxime and kanamycin on tomato cotyledonary leaf explants of BR3. In left column cefotaxime, **A**. Control (0 mg/l), **B**. 50 mg/l, **C**. 100 mg/l, **D**. 150 mg/l and **E**. 200 mg/l; in right column kanamycin, **F**. Control (0 mg/l), **G**. 7.5 mg/l, **H**. 12.5 mg/l, **I**. 25 mg/l and **J**. 50 mg/l (Photographs were taken 30 days after inoculation).

Agrobacterium culture density and infection time on transgenic efficiency with vector pBI121, pK7WG2_AtNHX1_1.6, pK7WG2_OsNHX1_1.6

Agrobacterium optical culture density and infection time are crucial parameter for transformation experiments. Firstly, the 5 tomato varieties were subjected under a set of optical density ranged from 0.6-1.4, with 2 different incubation periods 15 and 30 min with *Agrobacterium* vector *pBI121*, *pK7WG2_AtNHX1_1.6,* and *pK7WG2_OsNHX1_1.6* (Table 4). Among different combinations tested, the maximum GUS positive explants (95%) and germination efficiency (96%) were obtained at OD_{600} 1.1-1.4 with 30 min of infection period (Fig. 4). However, pricking of seeds lead to lower germination efficiency after transformation with the *Agrobacterium* vector *pBI121* in 5 tomato varieties. Thus, non-pricked seeds were preferred for this study (17).

Table 4. Effect of incubation time and culture density on *in planta* transformation

Tomato varieties	Incubation time (min)	OD_{600}	*Agrobacterium* vector *pBI121*	*Agrobacterium* vector *pK7WG2_AtNHX1_1.6*	*Agrobacterium* vector *pK7WG2_OsNHX1_1.6*
			GUS % of transformed seed	Germination efficiency (%)	Germination efficiency (%)
BR2	15	0.6-1.0	62	82	77
		1.1-1.4	85	85	82
	30	0.6-1.0	77	86	80
		1.1-1.4	92	94	86
BR3	15	0.6-1.0	65	78	80
		1.1-1.4	81	83	84
	30	0.6-1.0	78	81	85
		1.1-1.4	95	92	90
BN2	15	0.6-1.0	66	77	83
		1.1-1.4	82	82	84
	30	0.6-1.0	72	85	89
		1.1-1.4	94	96	94
BN3	15	0.6-1.0	74	75	82
		1.1-1.4	83	81	84
	30	0.6-1.0	80	79	89
		1.1-1.4	90	92	93
Bh	15	0.6-1.0	62	82	62
		1.1-1.4	72	84	72
	30	0.6-1.0	66	89	66
		1.1-1.4	86	93	86

Values were presented from three independent experiments, each with 50 seeds for each treatment. Randomly selected 5 cotyledons were assayed for every GUS expression assessment. It was calculated from the percentage of the surviving explants.

Assessment of co-cultivation period on in planta transformation

A range of co-cultivation periods (24-72 hr) were evaluated for obtaining maximum transgenic efficiency in tomato, keeping OD_{600} 1.1-1.4 and infection time of 30 min constant. Here, comparatively faster germination of seeds was observed with co-cultivation time of 72 hr than the seeds which were co-cultivated for 24 hr (Table 5). Despite giving faster germination with 72 hr of co-cultivation, the putative transformed plants gave a lower survival rate during acclimatization. Based on varied survivability, co-cultivation for 24 hr was noted as the optimum time required for *in planta* transformation. Furthermore, all the varieties acted better with *Agrobacterium* vector *pK7WG2_AtNHX1_1.6* than *Agrobacterium* vector *pK7WG2_OsNHX1_1.6* except BN3 (Table 5).

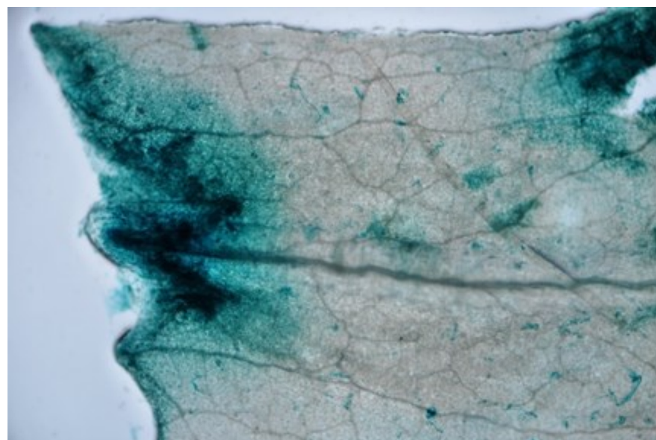


Fig. 4. GUS expression (blue color) of the tissue taken from putative tomato seedlings.

Table 5. Effect of co-cultivation period on putative transformants

Tomato varieties	Co-cultivation period (hours)	***Agrobacterium* vector *pBI121***		***Agrobacterium* vector *pK7WG2_AtNHX1_1.6***		***Agrobacterium* vector *pK7WG2_OsNHX1_1.6***	
		Mean no. of germinated seeds ±SD	**Germination efficiency (%)**	**Mean no. of germinated seeds ±SD**	**Germination efficiency (%)**	**Mean no. of germinated seeds ±SD**	**Germination efficiency (%)**
BR2	24	45±1.15	90	44±1.15	88	44±2.08	88
	48	27±1.52	54	40±1.52	80	43±0.57	86
	72	44±0.58	88	45±1.21	90	40±1.15	80
BR3	24	42±1.42	84	46±1.51	92	47±2.52	94
	48	22±1.13	44	45±1.34	90	45±1.13	90
	72	42±1.15	84	45±0.57	90	40±0.57	80
BN2	24	44±1.42	88	43±2.16	86	40±1.53	80
	48	42±0.58	84	46±2.44	92	47±1.15	94
	72	40±1.34	80	45±1.47	90	45±2.08	90
BN3	24	46±1.08	92	43±1.05	86	42±1.42	84
	48	35±2.16	70	43±2.16	86	43±1.14	86
	72	43±1.42	85	45±1.34	90	40±1.05	80
Bh	24	37±1.14	74	45±2.52	90	32±1.38	64
	48	15±1.52	30	08±2.47	16	15±1.41	30
	72	32±0.57	64	10±1.41	20	30±1.15	60

Values were presented from three independent experiments, each with 50 seeds for each treatment.

Acclimatization and putatively transformed seedling establishment

For successful development of regenerated plantlets in natural environment, full-grown rooted plantlets were transplanted to the soil in small paper pots and covered with pierced poly bags for adaptation (Fig. 5). During hardening maximum survival rate was obtained in BN2 (75%) while the lowest in Bh (40%) (Table 6). The survival rate of all varieties was found satisfactory when they were relocated to larger pots and shifted to net house (Fig. 5).

Fig. 5. Acclimatization and development of regenerated plantlets to the natural environment transformed with *Agrobacterium* vector in all five varieties. Mature plants infected with *pBI121*; **A**. BR2, **B**. BR3, **C**. BN2, **D**. BN3, **E**. Bh; Mature plants infected with *pK7WG2_AtNHX1_1.6*; **F**. BR2, **G**. BR3, **H**. BN2, **I**. BN3, **J**. Bh; Mature plants infected with *pK7WG2_OsNHX1_1.6*; **K**. BN2, **L**. BN3, **M**. Bh; **N**. flower and fruit setting in BN2; **O**. flowering in BN2.

Salinity stress tolerance test of tomato leaf discs from transformed tomato varieties

Leaf disk senescence assay of control and transformed plants was carried out to estimate salinity stress tolerance level of transformants. In control trials, faster bleaching was viewed at 100 mM NaCl (within 3 days), though no regular pattern was observed (Table 7). In contrast, leaf disks

Table 6. Survival rate of regenerated tomato plantlets following *in planta* transformation

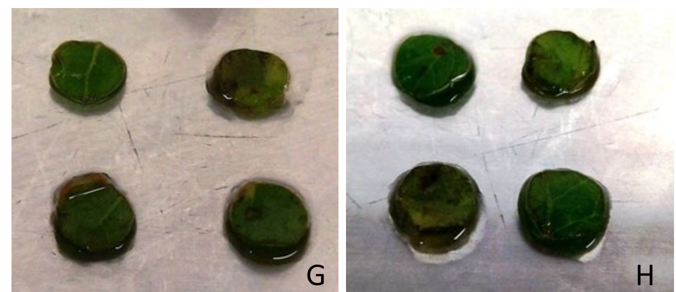
Tomato Varieties	*Agrobacterium* Vector *pBI121*			*Agrobacterium* vector *pK7WG2_AtNHX1_1.6*			*Agrobacterium* vector *pK7WG2_OsNHX1_1.6*		
	Survival during acclimatization (%)	No. of plants transferred to the soil	Natural Environment (%)	Survival during acclimatization (%)	No. of plants transferred to the soil	Natural Environment (%)	Survival during acclimatization (%)	No. of plants transferred to the soil	Natural Environment (%)
BR2	70	14	35	75	15	40	70	14	35
BR3	60	12	25	65	13	35	55	11	30
BN2	75	15	35	80	16	45	70	14	40
BN3	65	13	25	60	12	20	55	11	30
Bh	40	08	20	45	09	20	50	10	25

Twenty plants of each variety were taken for acclimatization.

Table 7. Bioassay of salinity stress tolerance of transformed seedlings

Salt concentrations		Leaf disks from control plant		Leaf disks from transformed plant	
(mM)	dS/m	Visual appearance	Time to bleach	Visual appearance	Time to bleach
0 mM	0 dS/m	Normal	Not bleached	Normal, Green	Not bleached
5 mM	0.5 dS/m	Bleached	14 days	Normal, Green	14 days
10 mM	1 dS/m	Bleached	12 days	Normal, Green	14 days
20 mM	2 dS/m	Bleached	9 days	Normal, Green	14 days
50 mM	5 dS/m	Bleached	5 days	Normal, Green	14 days
100 mM	10 dS/m	Bleached	3 days	Started to bleach	14 days

from transformed plants remain green at day 14 of the experiment up to 50 mM NaCl. Bleaching of disks seems to be initiated at 100 mM NaCl on day 14. The leaf disks remain typical visually within this duration; no sign of bleaching was observed (Fig. 6). Also, the rate of bleaching did not increase with the increase of NaCl concentration in liquid MS media. Besides, no relative impact of time on the rate of bleaching observed in these trials (Table 7).



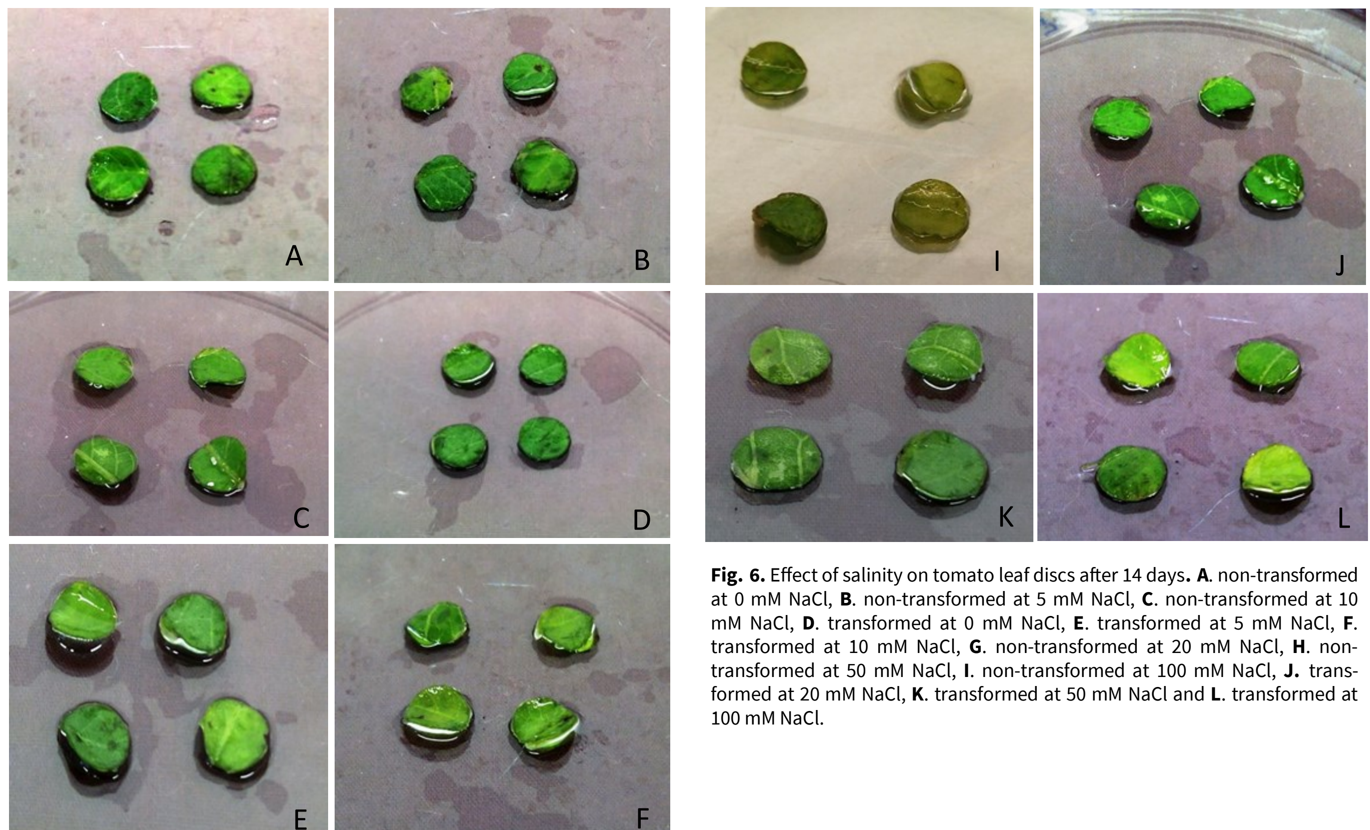


Fig. 6. Effect of salinity on tomato leaf discs after 14 days**. A**. non-transformed at 0 mM NaCl, **B**. non-transformed at 5 mM NaCl, **C**. non-transformed at 10 mM NaCl, **D**. transformed at 0 mM NaCl, **E**. transformed at 5 mM NaCl, **F**. transformed at 10 mM NaCl, **G**. non-transformed at 20 mM NaCl, **H**. non-transformed at 50 mM NaCl, **I**. non-transformed at 100 mM NaCl, **J.** transformed at 20 mM NaCl, **K**. transformed at 50 mM NaCl and **L**. transformed at 100 mM NaCl.

Discussion

This study shows the insertion of NHX1 gene in tomato plants improve tolerance to salt stress. Transgenic rice expressing NHX1 gene has been reported earlier for improved salt tolerance in Bangladeshi varieties (21-24). To our knowledge, improved salt tolerance in Bangladeshi tomato cultivars following *in planta* transformation has been reported here for the first time. Earlier, *in planta* transformation of tomato using fruit injection and floral dip was reported (11). Reports are on *in planta* transformation by smearing *Agrobacterium* culture on floral buds of tomato plant using a paintbrush (12).

In this study*, in planta* transformation experiment was performed on 5 varieties using mature seeds as explants with *pBI121, pK7WG2_AtNHX1_1.6* and *pK7WG2_OsNHX1_1.6* constructs to obtain transgenic salt-tolerant tomato varieties based on optimum conditions determined at initial steps performed with *pBI121.* Next, transgene expression was confirmed by GUS histochemical analysis and antibiotic selection. Finally, leaf disc senescence assay was performed to confirm salt tolerance of the first generation plants, which indicates transgene expression as well.

Based on our investigation, the non-pricked seeds found better GUS positive expression, while culture density of 1.0-1.2 OD_{600} with 30 min of incubation time gave the maximum percentage of GUS assay and better survival of explants in selection medium. Similar observation was also found in soybean, wheat and chickpea while performing *in planta* transformation (25-27).

This study has experienced co-cultivation time as the main factor influencing transformation. A gradual decrease of survival percentile was observed during increase of co-cultivation period. Thus, co-cultivation time span of 1 day (24 hrs) was found appropriate in transient GUS expression (86-100%) for the tested varieties. This observation agrees with an earlier report (9). Transformants were observed to have 90-95% efficiency by transient GUS expression (Fig. 4). Correspondingly, blue colored zones were absent in control plant tissues.

Leaf disk senescence assay of transformed and non-transformed plants was performed here as a bioassay for the estimation of salt tolerance potential. Present work shows that insertion and expression of the Na^+/H^+ antiporter gene in tomato plants improved tolerance to salt stress. After successful transformation, salt tolerance up to 10 dS/m till 14 days has been observed here. Therefore, this study represents a protocol that results in a sufficient number of transformants with improved salinity tolerance.

Previously, salinity treatment of 15 dS/m found to be detrimental for seed germination and seedling growth of BINA tomato 10 (tested on 0-15 dS/m of NaCl for 11 days) (26). In another report, 8 Bangladeshi tomato varieties were observed to have significantly lower yield per ha when grown on coastal land with salinity of 11-13 dS/m (27). In these studies held with Bangladeshi tomato cultivars, high yielding varieties has lost their production capacity due to salt stress but the approach to solve the issue has been based on adaptation rather than genetic improvement. At this point, our study differs from the others, as we focused on genetic modification of salt-sensitive variety for enhanced salt tolerance level whereas, the other reports focused on screening of moderately salt-tolerant varieties developed through breeding.

This study presents a basic comprehensive *in planta Agrobacterium*-mediated transformation protocol, which can be efficiently replicated for improving the climate resilience of other tomato varieties through successful introduction of the foreign gene like antiporter gene. Thereby tomato production could mitigate climate vulnerability and achieve better harvest to secure nutritional security.

Conclusion

The present study demonstrates an *Agrobacterium* mediated *in planta* transformation protocol, which provides a baseline for efficient insertion of gene into tomato cultivars. To our knowledge, this study pioneers for *in planta* transformation protocol of Bangladeshi tomato varieties for improved salt tolerance by inserting Na^+/H^+ antiporter gene. However, stable expression of target gene in progenies remained to be accomplished. Based on the salinity level increase in the cultivable area of Bangladesh and nearby countries, this study was a timely attempt which can be considered as a foundation for future approaches to improve salinity tolerance in tomato.

Acknowledgements

This research was supported by BAS-USDA-PLAS-BRACU-Li03 (Tomato) under Bangladesh Academy of Sciences and USDA Endowment Fund. The authors are grateful to Professor Zeba Islam Seraj, Department of Biochemistry and Molecular Biology, University of Dhaka; Samsad Razzaque, Doctoral fellow, Department of Integrative Biology, University of Texas, Austin, U.S.A. Also, the authors like to thank correspondents from Bangladesh Agricultural Research Institute (BARI) and Bangladesh Institute of Nuclear Agriculture (BINA) for providing plant materials.

Authors contributions

All authors have contributed equally in framing the manuscript.

Compliance with ethical standards

Conflict of interest: None

Ethical issues: None.

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