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Synergistic effect of C2H2 type Zinc-finger protein with LEA promoter to enhance abiotic stress tolerance in *Brassica juncea*

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Abstract

The Cys2/His2 (C2H2) type zinc finger (ZF), and Late-embryogenesis abundant (LEA) proteins are associated with various cellular processes that play an important role in plant development and abiotic stress tolerance. The study was designed to evaluate the role of P_{LEA1}:*Bc*ZF1 to enhance abiotic stress tolerance in *Brassica juncea*. The Group 4 LEA, LEA4-1, and ZF proteins isolated from *B. napus* and *B. carinata* respectively; were expressed in *B. juncea* cv. varuna. Expression of ZF protein in *B. juncea* under the control of LEA promoter showed increased tolerance against multiple abiotic stresses: salt, oxidative and drought. The increased level in the stability of total cellular membrane was observed in the transgenic lines (ZL1, ZL2 and ZL4) of *B. juncea*. The phenotypic analysis of transgenic lines also showed increased level of root and shoot length as compared to wild type (WT) plants under abiotic stresses. Our study suggest that cDNA encoding *BcZF1* and the promoter LEA1 function as regulatory molecules involved in stabilizing and modulating the optimal plant growth under various abiotic stresses.

Keywords: abiotic stress, *Brassica juncea*, *B. napus*, *B. carinata*, C2H2-ZF1 (Zinc finger), drought stress, LEA, oxidative stress, salinity stress, transgenic.
Abbreviations: Abscisic acid (ABA); CMS-cell membrane stability; LEA protein-Late Embryogenesis Abundant protein; MS medium-Murashige and Skoog medium; REC-relative electrical conductivity; RT-PCR- real-time polymerase chain reaction; TF-transcription factor; WT-wild type; ZF1-zinc finger 1

Introduction

Biotic and abiotic stresses can severely affect plant growth and productivity. Plants when exposed to stress conditions take some adaptive responses to survive and achieve optimal growth. Adaptive response in the form of transcriptional modulation is one of the important strategies to overcome the imposed stress (Tran and Mochida, 2010). Various *Cis*-element transcription factors bind to the promoter of stress responsive genes (Cramer et al., 2011) that are involved in ameliorating different stresses (Yamaguchi and Shinozaki, 2006). The Dehydration Responsive Elements (DRE), Ethylene Responsive Factor (ERF) WRKY, Myeloblastosis (MYB) and basic helix-loop-helix (bHLH) transcription factors (TFs) play important roles in stress signalling (Shinozaki et al., 2003). Similarly, one class of transcription factor, C2H2-type ZF protein with conserved (C2H2) domain (Ciftci et al., 2007) in the eukaryotes helps to cope with abiotic stresses (Gourcilleau et al., 2011; Jiang and Pan 2012; Martin et al. 2012). Several *Arabidopsis* C2H2 type ZF proteins have been reported to function as transcriptional repressors and moderate the abiotic stress effects in plants (Sakamoto et al., 2004). Moreover, various regulatory genes belong to C2H2 family including Zat12 are thought to be involved in cold and oxidative stress response (Chinnusamy et al., 2007; Davletova et al., 2005). The C2H2 type ZF protein isolated from rice was over-expressed into tomato that showed an enhanced tolerance against abiotic stresses (Mukhopadhyay et al., 2004). Similarly, the novel cold inducible ZF protein from soybean (SCOF-1) conferred cold tolerance in *Arabidopsis* plants (Kim et al., 2001). Likewise, the ZF protein isolated from *Solanum lycopersicum* improved cold tolerance in transgenic *Arabidopsis* and rice (Zhang et al., 2011). Recently, the over-expressed lines of *Arabidopsis* expressing ZF proteins (*AtC3H49/AtTZF3* and *AtC3H20/AtTZF2*) conferred ABA hypersensitivity that helped the plants to reduce transpiration, and thus improved drought tolerance (Lee et al., 2012). The aforementioned studies demonstrated the role of ZF protein under the control of constitutive promoter (CaMV 35S). However, in the present study, we have used the functionally characterized LEA4-1 promoter. Previously, our lab had demonstrated the function of LEA4-1 promoter, which played crucial role against abiotic stress tolerance (Dalal et al., 2009). LEA protein has also been reported to be involved in the desiccation tolerance during later stages of seed development, and was termed as Late-Embryogenesis Abundant (LEA) proteins (Dure et al., 1989). Most of the LEA proteins are hydrophilic in nature (Garay et al., 2000), and are classified into different members from Class 1 to 9 based on amino acid

sequence or different sequence motifs (Battaglia et al., 2008; Hundertmark and Hincha, 2008). There are several evidences which support an increase in accumulation of LEA protein in vegetative tissues after an exposure to water deficit environment (Olvera et al., 2010). Among different groups of LEA, group4 LEA1 has been induced by abscisic acid (ABA), salt, cold and osmotic stresses in *Brassica* leaf tissues (Dalal et al., 2009), whereas the over expression of this protein in *Arabidopsis thaliana* conferred tolerance against various abiotic stresses. The over expression of group 4 and 2 (RAB16A) LEA1 proteins in indica rice has conferred salinity tolerance (Ganguly et al., 2012). In addition, it was also demonstrated that the mutant of LEA1 *Arabidopsis* for group 1 LEA protein expressed dehydration induced phenotype earlier than WT plants (Manfre et al., 2006). However, mechanistic function of this LEA protein is still vague, although it has been identified that the LEA4 proteins have metal binding affinity and may be involved in reducing the oxidative damage induced by abiotic stress (Liu et al., 2011). Therefore, the present study was fragmented into two parts; the first with the objectives to isolate the desired C2H2-ZF protein 1 (ZF1) from *B. carinata*, and group 4 LEA1 promoter from *B. napus.* The second part was done to develop transgenic lines of *B. juncea* with desired construct, and their phenotypic evaluation against multiple abiotic stresses including salinity, oxidative and drought.

Results

Plant transformation and regeneration

Floral dip transformations were carried out using *Agrobacterium* cells harbouring pBinAR-P_{LEA1}: *BCZF1* (Fig 1A). The seeds were harvested from the plants, which were subjected to floral dip transformation, and screened in Murashige and Skoog (MS) medium supplemented with kanamycin (25 mg l^{-1}). The results revealed that the leaves of kanamycin resistant plants were green in colour, while the susceptible shoots turned bleached. However, the transformation efficiency *Agrobacterium* mediated floral dip transformation was 1.5% (data not presented). Four independent transgenic lines (ZL1, ZL2, ZL3 and ZL4) were used in molecular and phenotypic analysis.

Genomic PCR

The results of genomic PCR of putative transformants of T_1 plants ensured the successful transformation of *B. juncea* (Fig 1B), which demonstrated no amplification of the expected size for *npt*II (700 bp) in the WT (lane 2). Genomic PCR amplification produced a desired band as expected in all the four transgenic lines (ZL1, ZL2, ZL3 and ZL4) which corresponds to *npt*II selection marker gene.

RT-PCR analysis

The results of RT-PCR confirmed that the WT plant (Lane 2) was not able to generate an amplification product of 700 bp, whereas transgenic lines produced the desired amplification (700 bp) which conferred *npt*II gene (Fig 1C). The T_1 seeds harvested from transgenic *B. juncea* lines were identified as transformed with P_{LEA1}:*BCZF1*. Therefore, the subsequent plant experiments were performed using these transformed T_1 seeds.

Phenotypic evaluation of transgenic plants under abiotic stresses

Salinity stress and Cell membrane stability

Transgenic *B. juncea* lines (T_1) were exposed to the high salinity stress using MS medium supplemented with 300mM NaCl, and the results indicated enhanced growth with normal shoots and roots (Fig. 2A) as compared to the WT (untransformed controls). Under salinity stress conditions, the growth of roots and shoots was stunted in the WT individuals. After 15 days of exposure to salinity stress, transgenic lines of *B. juncea* showed longer roots (13.4 cm) and shoots (4.6 cm) as compared to WT (1.4 cm roots and 0.7 cm shoots) [Fig. 3A (roots) and 4A (shoots)]. The differences in cell membrane stability (CMS) with respect to ion leakage were also observed between the transgenic plants and WT during salinity stress. The CMS of transgenic lines was significantly higher than WT (Fig. 5). The ion leakage of transgenic plants was measured ranging from 30-39%. On the other hand, the WT plants showed very high ion leakage (approximately 55%). These findings suggested that the transgenic lines could be less damaged than the WT plants, when exposed to high salinity stress.

Oxidative stress

The exposure of transgenic lines of *B. juncea* to oxidative stress using MS medium supplemented with 4 mM H_2O_2 indicated prominent root and shoot growth as compared to the WT plants. Interestingly, no roots and shoots were developed in the WT plants (Fig. 2B). After an exposure to oxidative stress for 15 days, the growth of roots and shoots was measured, and transgenic lines of *B. juncea* exhibited average root length of 14.4 cm as opposed to WT plants that showed redundant growth of 1.5 cm only (Fig. 3B). The similar results were obtained with respect to shoot growth where transgenic lines displayed an average shoot size 4.7 cm as compared to 0.8 cm in the WT (untransformed) plants (Fig. 4B).

Drought stress and Relative Water Content (RWC)

Under water stress, relative water content (RWC) of *B. juncea* transgenic lines ranged from 42-50%, while it was only 32% in WT (Figure. 6B). The data indicated that water retention capacity/turgor pressure of the transgenic lines were quite stable as compared to WT (Fig. 6A). After 15 days of continuous water stress, leaves of control plants (wild type) wilted and dropped off as compared to the transgenic plants (Figure 6A). The experimental transgenic lines were irrigated after 2 weeks of water stress imposition, and all transgenic lines recovered well and exhibited normal phenotypic appearance (data not presented), whereas the wild type *B. juncea* didn't recover at all. Moreover, the flowering time, silique formation and seed setting were also on time in the transgenic lines (Figure 6A). We also recorded yield and found that transgenic seeds from one of the transgenic line (ZL4) yielded 1.30 g, whereas no yield (0g) was obtained in WT plants under drought stress (data not presented).

Discussion

In this study, we explore the collective effect of ZF protein (ZF1) isolated from *B. carinata* driven by LEA1 promoter in transgenic lines of *B. juncea* for abiotic stress tolerance.

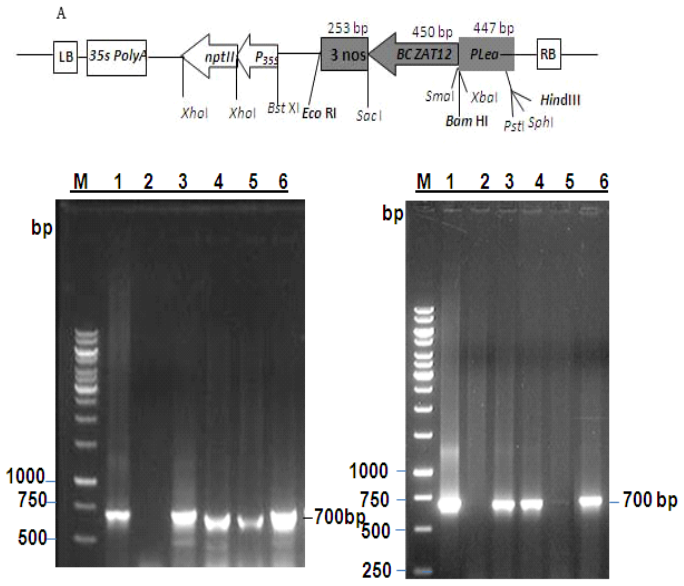

Fig 1. Schematic illustration of T-DNA regions containing P_{LEA1}:*BCZF1*. (A) cloned into the binary vector pBinAR contains nos-nptII, *neomycin phosphotransferase* II gene under control of nopaline synthase promoter; p35S, 35S promoter of cauliflower mosaic virus; (B) Genomic PCR amplification of nptII kanamycin resistance gene. An amplification product of nptII (700 bp) was present in plasmid pBinAR:: PLEA1:BCZF1 (lane 1) which serves as a positive control and no amplicon was observed as expected in wild type (untransformed) plant (lane 2). Transgenic *Brassica* lines ZL1 Zl2, ZL3 and ZL4 (lane 3-6) produced an amplification product of the expected size (C) RT-PCR , the wild type *Brassica* line did not generate an amplification product, while the amplication of transgenic lines (ZL1, ZL2,ZL3 and ZL4) produced a 700 bp band.

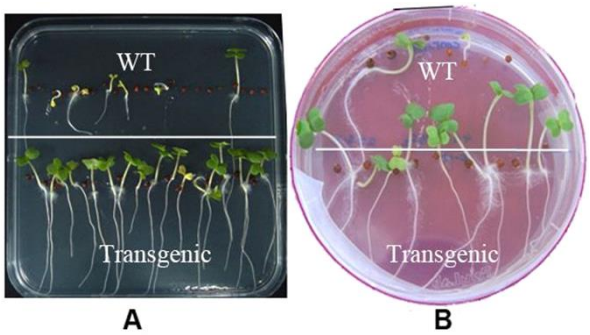

Fig 2. The phenotypic growth expression of transgenic expressing P_{LEA1}:*BCZF1* and wild type *B.napus* on (A) MS medium supplemented with 300 mM NaCl, the upper panel showed wild type and the lower panel showed the growth of transgenic plants (B). MS medium supplemented with 4mM H_2O_2, where the upper panel showed wild type and lower panel showed transgenic plants.

The functional and phenotypic evaluation of transgenic *B. juncea* (P_{LEA1}:*BCZF1*) lines were extensively evaluated for abiotic stresses including salinity, oxidative and drought. The gene integration and transcription were confirmed by PCR (Fig. 1B) and RT-PCR (Fig. 1C) analysis using *npt*II gene, which encode for kanamycin resistance in plant. The physiological assessments of the transgenic lines were observed under certain parameters which include the salinity, drought and oxidative stresses. The transgenic lines showed significant tolerance against salinity stress as compared to the WT plants of *B. juncea* in vivo (Fig. 2A). Our findings are in agreement with the previously published studies where, the ZF protein isolated from rice (OSISAP1) conferred salinity tolerance in the transgenic lines of tomato (Mukhopadhyay et al., 2004), and the P_{LEA1}:*BCZF1* significantly increased the level of drought tolerance in tomato plants (Rai et al., 2013). The cell membrane integrity of the transgenic *B. juncea* under salinity stress was tested against the WT plants. The CMS estimates by ion leakage have been widely used to differentiate between stress-tolerant and susceptible cultivars in crops (Blum and Ebercon, 1981). Abiotic stresses in plants initially damage the structure of the membrane, thereby, affecting its function and lead to an increase in membrane permeability, and ultimately results in leakage of the intracellular contents (Jia et al., 2002). In this study, the relative electrical conductivity of the transgenic lines after an exposure to salt stress was lower than those of non-transgenic lines of *B. juncea* (Fig. 5). Therefore, it is evident from the above fact that expression of ZF1 is controlled by LEA1 promoter that maintains the stability of cell membrane in transgenic *B. juncea* under salinity stress. However, the previous studies have shown a direct link of the constitutive expression of ZF protein (*AZF1* and *AZF2*) with enhanced salinity tolerance with compromising the growth of *Arabidopsis* plant (Vogel et al. 2005). It has been speculated that ZF proteins have transcriptional repressing activity and act as negative regulator for plant growth when expressed constitutively (Kodaira et al., 2011). In contrast, we observed that the growth of transgenic plants was not suppressed in terms of total biomass as compared to WT plants under stress (Figure. 6A), which, therefore, reflected the positive synergistic effect and role of LEA1 promoter to drive the ZF protein. Moreover, the transgenic plants when exposed to oxidative stress, showed significant tolerance as compared to the WT plants (Fig. 2B, 3B and 4B). The results of oxidative stress also suggested that ZF1 along with the LEA promoter may mediate the action against oxidative stress. Hence, prominent expression of Zat12 and Zat7 cells under oxidative stress (H_2O_2), heat shock and/or wounding has been reported as well (Rizhsky et al., 2004). Furthermore, enhanced osmotic and oxidative stress tolerance of the transgenic plants expressing these genes has also been documented (Mittler et al., 2006). The growth of transgenic lines (ZL1, ZL2 and ZL4) were developmentally advanced and showed enhanced tolerance after an exposure to drought stress as compared to the WT *B. juncea* plants (Fig. 6A). Similar role of ZF transcription factor in the regulation of stomata has been demonstrated that protected the plants against abiotic stress (Huang et al., 2009). Moreover, increased water retention capacity of transgenic lines during abiotic stress supported the above facts whereby the expression of *BcZF1* gene by LEA1 promoter had a strong and synergistic effect in maintaining the RWC in leaves, which was achieved by preventing water loss from leaves. The ZF protein (*Bc*ZF1) isolated from *B. carinata* along with the LEA1 promoter

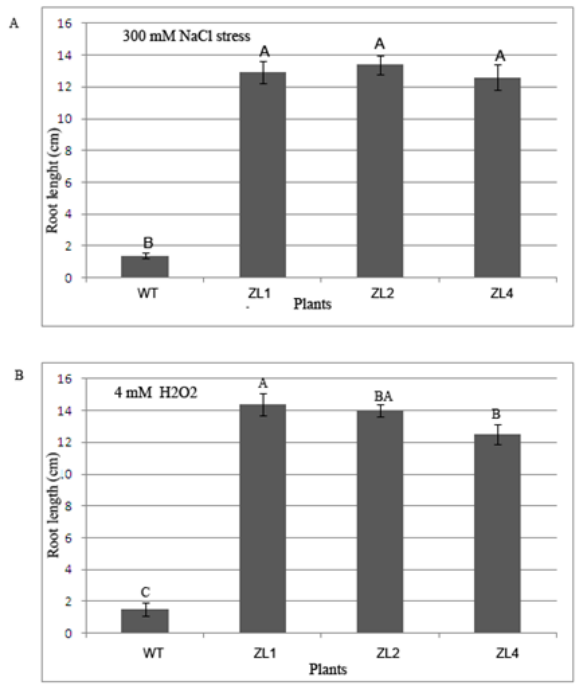

Fig 3. The root length of transgenic *B. napus* expressing P_{LEA1}:*BCZF1* and wild type plants (A) seedlings were grown in MS medium supplemented with 300 mM NaCl (B) seedlings grown in MS medium supplemented with 4mM H_2O_2 The error bars in the figure denote the standard deviation different letters above bars indicate significant differences according to ANOVA analysis by SAS (P<0.01).

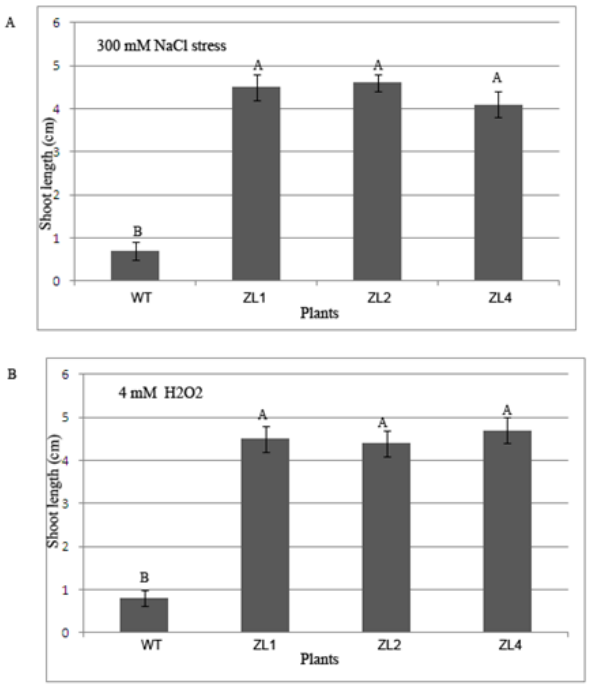

Fig 4. The shoot length of transgenic *B.napus* expressing P_{LEA1}:*BCZF1* seedlings grown in MS medium supplemented with (a) 300 mM NaCl (b) 4mM H_2O_2

from *B. napus* when expressed in *B. juncea* showed synergistic effect of enhance tolerance against several abiotic stresses. However, further study on the evaluation of these transgenic lines in field conditions will broaden the scope of ZF protein and LEA1 promoter to be used in breeding programs to develop cultivars for abiotic stress tolerance. In addition, it will be more interesting to know the regulatory pathway or gene interaction of ZF protein and LEA1 promoter. These further studies will enhance our understanding regarding the *ZF1* gene and LEA1 promoter to improve the crops adaptability against abiotic stresses. In conclusion, abiotic stress tolerance of transgenic lines strongly suggests that P_{LEA1}:*BCZF1* function as a stress regulator for plants under stress. The transgenic expression of C2H2-ZF protein along with the LEA1 promoter indicates a possible route for crop improvement against abiotic stresses. Our findings would help to lead an improvement of *Brassica* crops using the ZF protein and the LEA1 promoter to stabilize and maintain optimal plant growth under abiotic stresses.

Material and Methods

Seed sterilization and plant growth conditions

Seeds of *B. juncea* cv. varuna were obtained from Indian Agricultural Research Institute, Pusa, New Delhi. Seed sterilization, seeding and incubation for germination was followed as described by (Dalal et al., 2009). All plants including WT and transgenic were grown under controlled environmental conditions, maintaining 16 h photoperiod (light intensity of 130 µmol $m^{-2}s^{-1}$) and a day (21± 2ºC), night (18 ± 2ºC) cycle.

Construction of Binary construct pBinAR-PLEA1:BCZF1 preparation and plant transformation

The ZF1 was isolated by PCR amplification employing primers (forward primer ZF1-5'ATGGTTGCTATTTCAG-AGATC3' and reverse primer ZF1- 5'TCAACAAACAGG-TCTTCCAAG3') from *B. carinata* (DQ166621) and the LEA1 (AY766378) promoter was isolated from *B. napus*. The ZF1 protein and LEA1 promoter were submitted to NCBI gene bank with accession numbers: DQ166621 (ZF1) and AY766378.1 (LEA1). Zinc finger transcription factor (ZF1) was cloned into the binary vector pBinAR flanked by LEA1 promoter and the nos terminator (Fig. 1A). The cDNA of ZF transcription factor *BcZF1* is driven by abiotic stress-inducible promoter. LEA1 was cloned into the binary vector pBinAR using *Hind*III and *Eco*RI restriction sites (Fig.1A). The *Agrobacterium* strain GV-3101 harbouring the plasmid pBinAR-P_{LEA1}:*BCZF*1 was used for plant transformation using floral dip method of *B. juncea* (Verma et al. 2008). The putative transformants were screened on agar plates containing ½ x MS medium (Murashige and Skoog 1962) supplemented with kanamycin (50 mg ml^{-1}). The plates were kept in controlled environment room with 16 h photoperiod (light intensity of 130 µmol $m^{-2}s^{-1}$) and a day (21± 2ºC), night (18 ± 2ºC) cycle

Genomic PCR

To determine the successful integration of the pBinAR-P_{LEA1}:*BCZF1* into the genome of transgenic *Brassica*, genomic DNA was isolated from leaves of WT and putative transgenic *B. juncea* using C-TAB method (Xin and Chen, 2012). The *npt*II kanamycin selection marker primers were

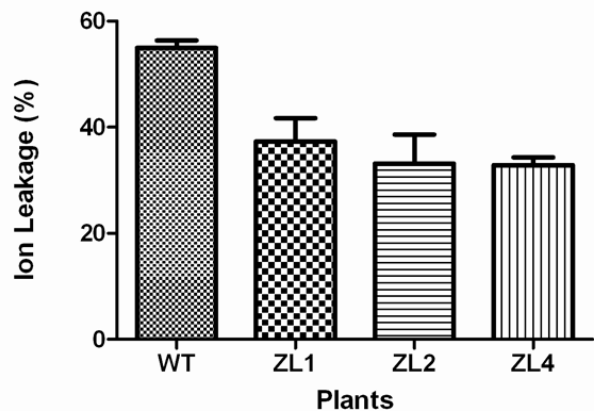

Fig 5. The percentage (%) cell membrane stability in the leaf discs of wild type and transgenic lines of Brassica juncea after 150mM NaCl treatment for 24h. Data is shown as bar graph which represents the each replicate where (n=9), horizontal lines represent the mean value. The data was analyzed using unpaired t-test at P<0.01.

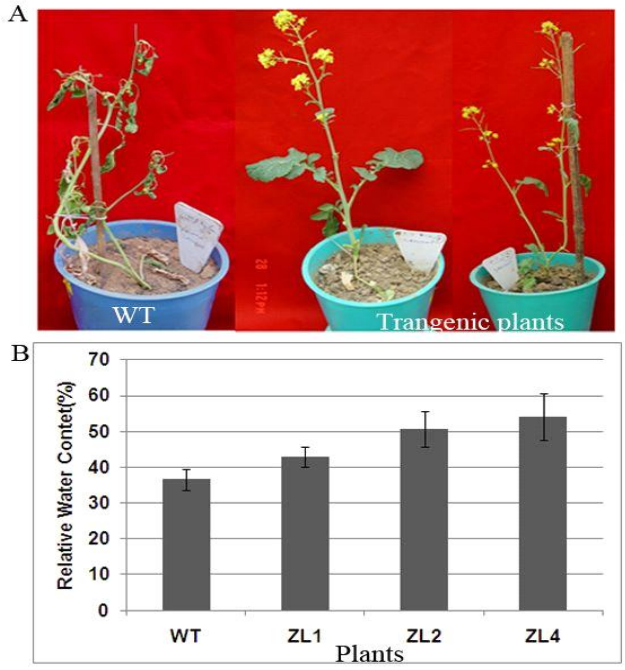

Fig 6. (a) Representative image of the transgenic expressing P_{LEA1}:*BCZF1* and the wild type *B.juncea* under water stress after two weeks. (b) Percentage (%) relative water content (RWC) of the transgenic *B. juncea* compared with the wild type *B. napus* transgenic after one weeks of water stress. The data was analyzed using unpaired t-test at P<0.01.

used for PCR reaction. The primers of nptII forward (5'CAATCGGCTGCTCTGATGCCG3') and reverse (5'AGGCGATAGAAGGCGATGCGC3'), were used for PCR analysis. The genomic PCR of total volume 25 µl, contained 10 ng of isolated genomic DNA, 1 mM dNTP, 0.5 mM of each primers, 0.5 unit of Taq polymerase and standard Taq polymerase buffer (New England Biolabs, USA). PCR cycling conditions consisted of an initial denaturation at 94^{o}C for 5 min; 35 cycles of 94^{o}C for 45 s, 58^{o}C for 45 s and 72^{o}C for 1 min; and a final extension at 72^{o}C for 10 min. PCR products were analyzed by 1% (w/v) agarose gel electrophoresis.

Reverse-transcriptase (RT)-PCR

To determine the translation of the inserted chimeric construct, total RNA was isolated from leaves of the plants, using the RNeasy Mini kit (Qiagen, USA) and treated with RNase-free DNase (Qiagen, USA) to remove contaminating DNA. The transgenic lines, which scored positive in PCR analysis, were analyzed by RT-PCR. The cDNA was synthesized from 500 ng of RNA using Qiagen RT-PCR kit, USA and used as template in PCR reaction after RT.

The *npt*II kanamycin selection marker primers were used as described in the earlier section. The total reaction mixture (25 µl) of RT-PCR contained 1µl of synthesized cDNA, 1 mM dNTP, 0.5 mM of each primers, 0.5 unit of Taq polymerase and standard Taq polymerase buffer (New England Biolabs). PCR cycling conditions consisted of an initial denaturation at 94^{o}C for 5 min; 35 cycles of 94^{o}C for 45 s, 60^{o}C for 45 s and 72^{o}C for 1 min; and a final extension at 72^{o}C for 10 min. PCR products were analyzed by 1% (w/v) agarose gel electrophoresis.

Phenotypic evaluation of Transgenic Brassica

Phenotypic and physiological evaluations of developed transgenic lines (ZL1, ZL2, and ZL4) along with WT (wild type) plants of *B. juncea* were performed. These phenotypic evaluations include salinity, oxidative and drought stress responses which was performed with the collected seeds of transgenic *B. juncea* expressing P_{LEA1}:*BCZF1*, and compared with WT (untransformed) plants.

Response against salinity

To determine the salinity stress response of transgenic lines, seeds after sterilization were grown on MS medium supplemented with 300 mM NaCl containing 0.8% agar and 3.0% sucrose. The germinated seeds were scored by measuring differences between transgenic and WT lines of *B. juncea* with reference to the emergence of green cotyledons and roots, following two-weeks of exposure to the stress. The experiment was carried out with three biological and technical replicates.

Water stress tolerance assay

Drought and oxidative stress responses of the transgenic lines were determined using three different transgenic lines (ZL1, ZL2, and ZL4) from the transgenic plants of *B. juncea* along with the WT (untransformed) plants which served as the experimental control. The seedlings of the transgenic and WT plants were first grown under normal condition to the vegetative stages (2-3 weeks). Water stress was imposed on 3 weeks grown plants by first withholding irrigation for almost two weeks (15 days) and irrigated with water afterwards. The RWC was measured after one week of drought stress from transgenic plant (ZL1, ZL2, and ZL4) and wild type plants of *B. juncea*. The RWC was determined as described by (Goel et al. 2010). Relative water content was calculated using formula [$^{RWC\ (\%)\ =FW\_DW/TW\_DW\times 100}$]. The experiments were repeated three times with three biological replicates. The data of relative water content of transgenic plants were compared with WT plants and statistically analyzed.

Oxidative stress

The transgenic lines (ZL1, ZL2, and ZL4) were also tested for their tolerance towards oxidative stress. The transgenic seeds were exposed to 4mM H_2O_2 for two weeks on MS media supplemented with 0.8% agar and 3.0% sucrose (Katiyar et al., 2006). Plants were scored on the basis of emergence of green cotyledons and roots on transgenic seeds of *B. juncea and* compared to WT (untransformed) plants. All experiments for oxidative stress tolerance were also conducted with three biological and three technical replicates.

Cellular membrane stability analysis

The CMS was analyzed by measuring the total ion leakage of the leaves by relative electrical conductivity (REC) (Farooq and Azam, 2006; Fu et al., 2012). Leaf tissue was cut in segments of $1cm^2$ with cork-borer. Five leaf segments were then salt stressed for 24h with 150mM NaCl solution at room temperature (25°C±2). After the stress, the leaf segments were washed five times with distilled deionized water and then put into 15ml of distilled deionized water in a falcon tube (50ml) and shaken using a rotary shaker (170 rpm) at room temperature (25°C±2). Electrical conductivity (EC) was measured 1h later using an ion conductivity meter (Mettler, USA) and counted as (C_1). The tubes containing the segments were autoclaved for 20 min at 121°C and the conductivity of autoclaved tissue was measured after cooling at room temperature and counted as (C_2). Relative electrical conductivity was measured using formula $C_1 / C_2 \times 100$.

Statistical Analysis

Results of the experiments such as root and shoot length along with RWC were calculated and analyzed for statistical significance using the GLM procedure of SAS. Analysis of Variance (ANOVA) was done followed by Duncan's multiple-range test ($P < 0.01$) with SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). The error bars in the figure denote the mean standard deviation and the different letters above bars indicate significant differences among the treatments.

Conclusion

In this study, we demonstrate the importance of ZF protein and LEA1 promoter to enhance multiple abiotic stress tolerance, such as drought, salt and oxidative. The results presented support the fact that the transcription factor zinc finger 1 (ZF1) and LEA as promoter from *Brassica* species are involved in abiotic stress tolerance. The findings of this study will help to enhance grain yield of agricultural crops including *Brassica*.The aforementioned approach is adequate to improve the agronomical traits of field crops for abiotic stress tolerance.

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