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ORIGINAL ARTICLE

TaZnF, a C3HC4 type RING zinc finger protein from *Triticum aestivum* is involved in dehydration and salinity stress

Preeti Agarwal[1] · **Paramjit Khurana**[1]

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Abstract

C3HC4-type RING finger proteins represent one of the largest transcription factors in the plant kingdom. They are known to play crucial roles in various plant processes like regulation of growth and development, signalling network and abiotic stress etc. The paper attempts to explore and dissect the function of *TaZnF* in response to abiotic stress like drought and salinity. The gene expression studies revealed the possible role of *TaZnF* in drought and salt stress response. The expression pattern was found to be similar in root tissue under both drought and salt stress. It was found to increase with the increase in the duration of stress with maximum fold > 3 under drought and > 15 fold in response to salt stress at 8 h. *Arabidopsis* transgenics overexpressing *TaZnF* were raised to analyse how the changes in gene expression levels affect plant's response under stress conditions. *TaZnF* overexpression conferred increased tolerance to both drought and salt stress as measured by several assays examining their growth and development. The transgenics showed better growth in response to dehydration stress given by both mannitol and Polyethylene glycol (PEG). Further, the transgenics were found to survive when subjected to drought stress for 14 days in contrast to wild type. They also showed high yield under severe drought conditions for one month in comparison to the wild type plants. Similarly the transgenics were found to perform well under salt stress conditions in terms of increased fresh weight, better growth, higher chlorophyll accumulation, higher membrane stability and increased proline content. The expression level of both drought and salt stress related marker genes was found to be higher in transgenics in comparison to wild type plants. Thus these observations clearly indicate that *TaZnF* functions as a positive regulator of stress response, and can be used as a candidate gene to improve and enhance drought and salt stress tolerance of various crop plants.

Keywords C3HC4-type · Dehydration stress · Salt stress · *Triticum aestivum* · RING zinc finger

Abbreviations

YII	Effective photosynthetic efficiency
ETR	Electron transport rate
Fv/Fm	Photosynthetic efficiency
PEG	Polyethylene glycol
ZFPs	Zinc finger proteins

Introduction

Plants are often challenged by various environmental stresses which tend to inhibit their growth and development. Amongst these unfavourable environmental cues, drought and salinity are known to impose serious threat to plant growth, survival and productivity. Consequently, plants have established intricate defence mechanism to facilitate their survival and adaptation under these unfavourable conditions. Drought stress is known to reduce the crop productivity by 70% (Kaur et al. 2008; Akram et al. 2013; Lum et al. 2014), and this reduced productivity remains a major concern for wheat grown in both arid and semi-arid areas. Similarly, soil salinity is also one of the important agricultural problems in both arid and semi-arid regions in different parts of the world. Salinity is reported to affect almost every aspect of the plant physiology and biochemistry (Murphy and Durako 2003; Cuartero et al.

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✉ Paramjit Khurana
param@genomeindia.org

[1] Department of Plant Molecular Biology, University of Delhi, South Campus, Dhaula Kuan, New Delhi 110021, India

2006).These adverse conditions are known to induce the expression of wide range of genes including Zinc Finger Proteins (ZFPs) (Xiong et al. 2002; Bartels and Sunkar 2005; Islam et al. 2009).

Zinc Finger Proteins (ZFPs) are one of the best studied transcription factor families reported to participate in various growth and developmental processes and also participate in both biotic and abiotic responses (Cheung et al. 2007; Kam et al. 2007; Jung et al. 2013). The zinc-binding motifs of ZFPs vary extensively in their structure as well as in functions like DNA or RNA binding to protein–protein interactions (Laity et al. 2001). The C3HC4-type RING finger genes form a large gene family and have been well studied in both *Arabidopsis* and rice (Wu et al. 2014). According to previous studies, 1400 E3 ubiquitin ligases have been reported and 469 of these proteins have 477 RING domains, thus forming the third-largest gene family in *Arabidopsis.* Moreover, out of these 477 RING domains, 186 RING-HC type domains were recognized based on the occurrence of typical RING domain structure (Stone et al. 2005). RING-HC proteins in *Arabidopsis* have been reported to function in various processes like root development, photomorphogenesis, secretory pathways, chromatin remodelling in seed dormancy and expression of various drought stress responsive genes (Matsuda et al. 2001; Hardtke et al. 2002; Wang et al. 2006; Liu et al. 2007; Qin et al. 2008).

Wheat is the most important cereal crop as it is a staple diet for more than one-third of the world population (Abd-El-Haleem et al. 2009). In the present study, we attempt to characterize our previously identified heat induced C3HC4 type *TaZnF* (Traes_4BS_37569EA13.1; hereafter referred to as *TaZnF*) under dehydration and salinity stress (Agarwal and Khurana 2017).

Materials and methods

Plant material, growth conditions and stress treatments

Genotypes of *Triticum aestivum* L. cv. PBW343 and *Arabidopsis thaliana* ecotype Col0 were used in the current study. For wheat seedlings, seeds were sown in plastic trays and kept in growth chamber (Conviron®, Canada) maintained at 20 ± 1 °C; 16 h light: 8 h dark photoperiodic cycle. While for *Arabidopsis,* seeds were grown on half MS media and kept in growth room maintained at 22 ± 1 °C and 16 h light: 8 h dark cycle. For expression analysis of *TaZnF* in response to salt (250 mM) and air dehydration stress, 10-day-old seedlings of PBW343 were given stress exposures at different time points (0, 1, 2, 4, 6 and 8 h). To measure the germination rate of wild type and *Arabidopsis* transgenics under salt stress, seeds of both wild type and transgenics were grown on half MS media supplemented with 150 mM of NaCl. To further study the effect of salt stress on overall growth of the seedlings, 2 weeks old seedlings of both *Arabidopsis* transgenics and wild type were subjected to gradual salt stress treatment (200 mM NaCl for 5 days followed by 300 mM NaCl for next 16 days). Moreover, these plants under stress conditions were also used for expression profiling of salt stress related marker genes. To analyse the response under dehydration stress, seeds of both transgenics and wild type were grown on half MS media supplemented with different concentrations of PEG (Polyethylene glycol 2%, 3% and 4%) and also with different concentration of mannitol (100 mM, 200 mM and 300 mM). To monitor survival and overall growth under drought stress, 22-day-old seedlings of both wild type and *Arabidopsis* transgenics were grown in soilrite and then subjected to drought stress by not watering the plants for 14 days, and then followed by re-watering for the next 5 days. To further monitor the overall growth and yield under severe drought stress, seeds of both wild type and transgenics were grown for one month under normal conditions and were then subjected to drought stress by not watering the plants for one month and thereafter analysis was performed. These plants under stress conditions were also used for expression profiling of drought stress marker genes. A representation of plant stage, kind of stress, level of stress and parameters studied is provided in Table 1.

Expression analysis by Real-time PCR

Total RNA was isolated from 10-day-old seedling of PBW343 and *Arabidopsis* plants using the RNeasy plant mini kit (Qiagen, Germany). On-column DNaseI treatment was done to remove genomic DNA contamination. Two µg of total RNA was used to synthesize cDNA using the High Capacity cDNA Archive kit (Applied Biosystems, USA) and this was mixed with 200 nM of each primer and SYBR Green PCR Master Mix (Applied Biosystems). The primers were designed using Primer Express version 2.0 (PE Applied Biosystems, USA) using default parameters. Real-time PCR analysis was done using the ABI Prism 7000 Sequence Detection System and Software (PE Applied Biosystems) under the following set of conditions including melt curve and dissociation curve: 95 °C for 10 min, 95 °C for 30 s, 60 °C for 1 min, 72 °C for 1 s; 95 °C for 1 min, 60 °C for 30 s, 95 °C for 30 s and 40 cycles. Two biological replicate and three technical replicate was used for analysis. *Actin* (KX533928.1) was used as internal control. The relative expression was estimated using 2-ΔΔCT method. Primers used are listed in Supplementary Table S1.

Cloning and plant transformation

For cloning, a 756 bp fragment (cDNA) of *TaZnF* was amplified from pGEM-T Easy::*TaZnF* plasmid using Topo::*TaZnF*-F and *TaZnF*-R primers (Table S1). This amplicon was then inserted into pENTR/D-TOPO vector (Invitrogen) for making entry clone. This cassette was then moved into pMDC32 destination vector by LR-clonase reaction. Overexpression transgenics of *Arabidopsis thaliana* Col0 were raised by floral dip transformation (Clough and Bent 1998). For analysis, three independent transgenic lines from T3 homozygous seeds were used and the results here represent at least three replicates.

Estimation of chlorophyll fluorescence

PSII activity was calculated as per Krause and Weis (1991). Pulse amplitude modulation fluorometer (Junior-PAM chlorophyll fluorometer, H. Walz, Germany) was used to measure the modulated chlorophyll fluorescence emission from the upper surface of the leaf. For measuring the induction of fluorescence, leaves were initially kept in dark for 20 min. Measurements of different PSII parameters like maximum photosynthetic efficiency (Fv/Fm), effective photosynthetic efficiency (YII) and Electron Transport Rate (ETR) were measured from rosette leaves from ten plants per transgenic line for both transgenics and wild type seedlings.

Estimation of total chlorophyll

Chlorophyll amount was estimated according to protocol mentioned by Hiscox and Israelstam (1979). Leaf weighing approximately 0.05 g was taken from both wild type and transgenic plants in three different replicates followed by incubation in 5 ml of DMSO for 4 h at 65 °C in dark. Absorbance was then measured at 645 nm and 663 nm using spectrophotometer (Beckman DUTM 640B, Beckman Instruments Inc., USA). Chlorophyll contents were calculated by the given formula.

$$\text{Ch a} = [(12.3A_{663} - 0.86A_{645})xV]/X \times 1000 \times W$$

$$\text{Ch b} = [(19.3A_{665} - 3.6A_{643})xV]/X \times 1000 \times W$$

where V is the volume of DMSO (mL), X represents path length (1 cm) and W indicates fresh weight (gm).

Measurement of membrane stability

Cell membrane stability was measured according to Bajji et al. (2002). For this, seedlings from both wild type and transgenics were submerged in 5 ml of distilled water in test tubes and then were kept at 30 °C for 30 min. After incubation, electrical conductivity (C1) was measured and then these seedlings were further autoclaved (temperature of 121 °C and pressure 15 psi) for 15 min and C2 was measured. Cell injury was calculated using the equation: CMS = [1-(C1-C2)].

Proline estimation

Proline level was measured according to Bates et al. (1973). For this, tissue weighing approximately 100 mg was grinded in 1 ml of 3% sulphosalicyclic acid followed by centrifugation at 15,000 rpm for 15 min. Supernatant obtained was divided and 400 µl of glacial acetic and 400 µl of ninhydrin was added to it followed by incubation at 100 °C for 1 h. After incubation, reaction was stopped by keeping the reaction mix in ice. 800 µl of toluene was added to the sample and followed by vigorous vortexing. Supernatant thus obtained was transferred to new MCT and absorbance was recorded at 520 nm in a UV–visible spectrophotometer (U-2810 Spectrophotometer, Hitachi, Japan). Proline content was calculated using the given equation:

µmoles of proline/gm fresh wt
= (µg protein/ml × ml toluene 5)/115.5µg/µmoles × gm sample

Statistical analysis

Statistical analysis was performed by calculating the mean value and standard error for all replicates. Student's *t* test was performed to find out the significant difference between wild type and transgenic lines and *P* value of ≤ 0.05 was considered to be significant and represented by asterisk *.

Results and discussions

Expression profiling of *TaZnF* under dehydration and salt stress in wheat

To understand the role of *TaZnF* under abiotic stress, expression profiling of *TaZnF* was done by real-time PCR analysis. For this, 10-day-old seedlings of *Triticum aestivum* L. cv. PBW343 were subjected to air dehydration and salinity treatment for various time intervals. Similar expression pattern was observed in both root and shoot tissues under dehydration stress. The expression level was initially found to decrease followed by gradual increase in the expression level with increase in the duration of stress Fig. 1a, b. The expression level of *TaZnF* was almost similar in both the tissues till 6 h of stress. At 8 h of stress,

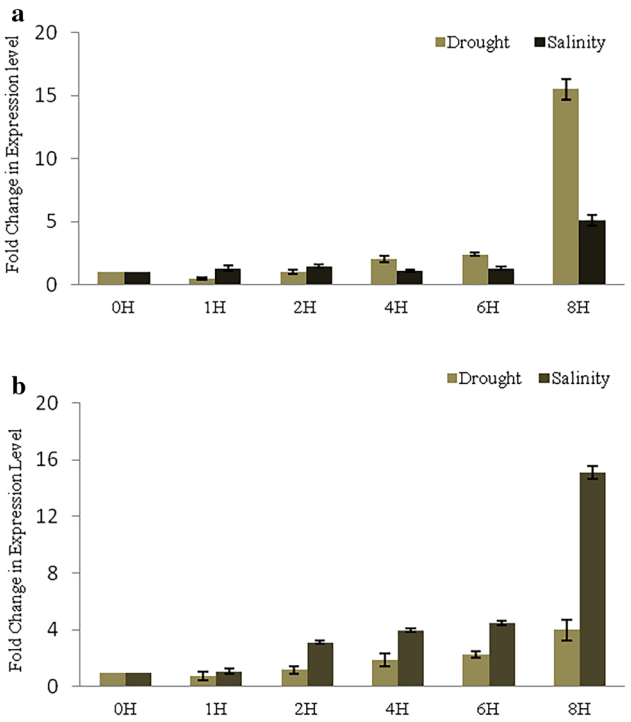

Fig. 1 Expression profiling of *TaZnF* transcription factor under dehydration and salt stress at different time points. Change in transcript abundance of *TaZnF* in response to dehydration and salt stress in **a** shoot and **b** in root tissue with increase in duration of stress. The expression level of control samples i.e. 0 h of treatment was normalized as 1.0. The results shown are the means ± SDs of at least three independent experiments

the expression was found to be higher in shoots (> 15 fold change) in comparison to roots (> 3 fold change). These results correlate with the expression pattern of CCCH zinc finger (BraA10g002330) in seedlings of *B. rapa* in response to mannitol stress (Pi et al. 2018). The expression level was found to decrease initially at 1 h of stress followed by further increase with the increase in duration of stress (3-9 h). Moreover, the expression of zf-C3HC4 in rice was also found to be strongly affected in leaf over root tissue of rice seedlings under drought stress (Minh-Thu et al. 2013).

The expression level was analysed in roots and shoot tissue under salt stress treatment (Fig. 1a, b). In roots the expression level was found to increase with increase in duration of stress and showed greater than 14 fold changes in expression level at 8 h of stress treatment. However in shoot tissue, the expression level increased gradually till 2 h followed by decrease at 4 h and 6 h and again increased at 8 h of stress. Such differential expression pattern has been reported for CCCH zinc finger proteins (BraA10g016510 and BraA08g018640) in seedlings of *B. rapa* under NaCl stress. Similarly RR-TZF (Arginine-rich motif-tandem CCCH Zinc Finger) of *B. rapa* like BraA09g046730 and BraA10g002330 showed increased expression within 3–6 h of stress, after which their expression was found to decline under salt stress (Pi et al. 2018). Another C3HC4 type zinc finger protein (BrRZFP1) from *B. rapa* was also found to be induced by salt treatment. The transcript level increased several fold after 30 min of stress treatment, reaching to a peak level after 8 h and then returning to its normal (Jung et al. 2013). Recent studies too have revealed that C2H2 zinc finger proteins act as key regulator of salinity and drought stress response (Wang et al. 2018). Another parallel observation was made for *AdZFP1* from *Artemisia desertorum* Spreng (induced in root, stem and leaf under drought stress as well as by salinity), *Arabidopsis* Zinc finger protein, *AZF2* and *STZ* (strongly induced in response to dehydration and high-salt stress) and AlSAP protein from *A. littoralis* (induced by salt, osmotic, heat and cold stress) (Yang et al. 2008; Sakamoto et al. 2004; Ben Saad et al. 2010). Thus, together these studies clearly indicate the probable role of *TaZnF* in both drought and salt stress as each of these processes were found to regulate the expression of *TaZnF*.

Overexpression of *TaZnF* confers enhanced tolerance under drought stress to transgenics

To find out the role of *TaZnF* under drought stress, seeds of both wild type and overexpression transgenics were grown on half MS media supplemented with different concentration of PEG (2%, 3% and 4%). PEG is often used to imitate desiccation in various dehydration based experiments (Premachandra and Shimada 1987). All the transgenic lines were found to grow well on all the different concentration of PEG than wild type plants. They displayed relatively longer root length and increased fresh weight (Fig. 2a–c). Earlier studies have documented that stress conditions results in overproduction of proline in plants which in turn imparts stress tolerance by maintaining the osmotic balance, stabilizing the membrane and also by preventing oxidative burst (Hayat et al. 2012). Therefore proline content was measured and it was found to be significantly higher in transgenics in comparison to wild type (Fig. 2e). Other parameters like membrane stability and chlorophyll content were also studied. Cell membranes are one of the foremost targets of many plant stresses and maintenance of its integrity and stability under water stress; this act as a key factor for enhanced tolerance under drought stress. Transgenics showed better membrane stability as estimated by the electrolyte leakage from cells (Fig. 2d). Under stress conditions, there is decrease in chlorophyll content due to damage done to the chloroplasts by active oxygen species (Hasegawa et al. 2000). Transgenics showed relatively higher chlorophyll content than wild type plants (Fig. 2f). Thus, the transgenics performed better under stress

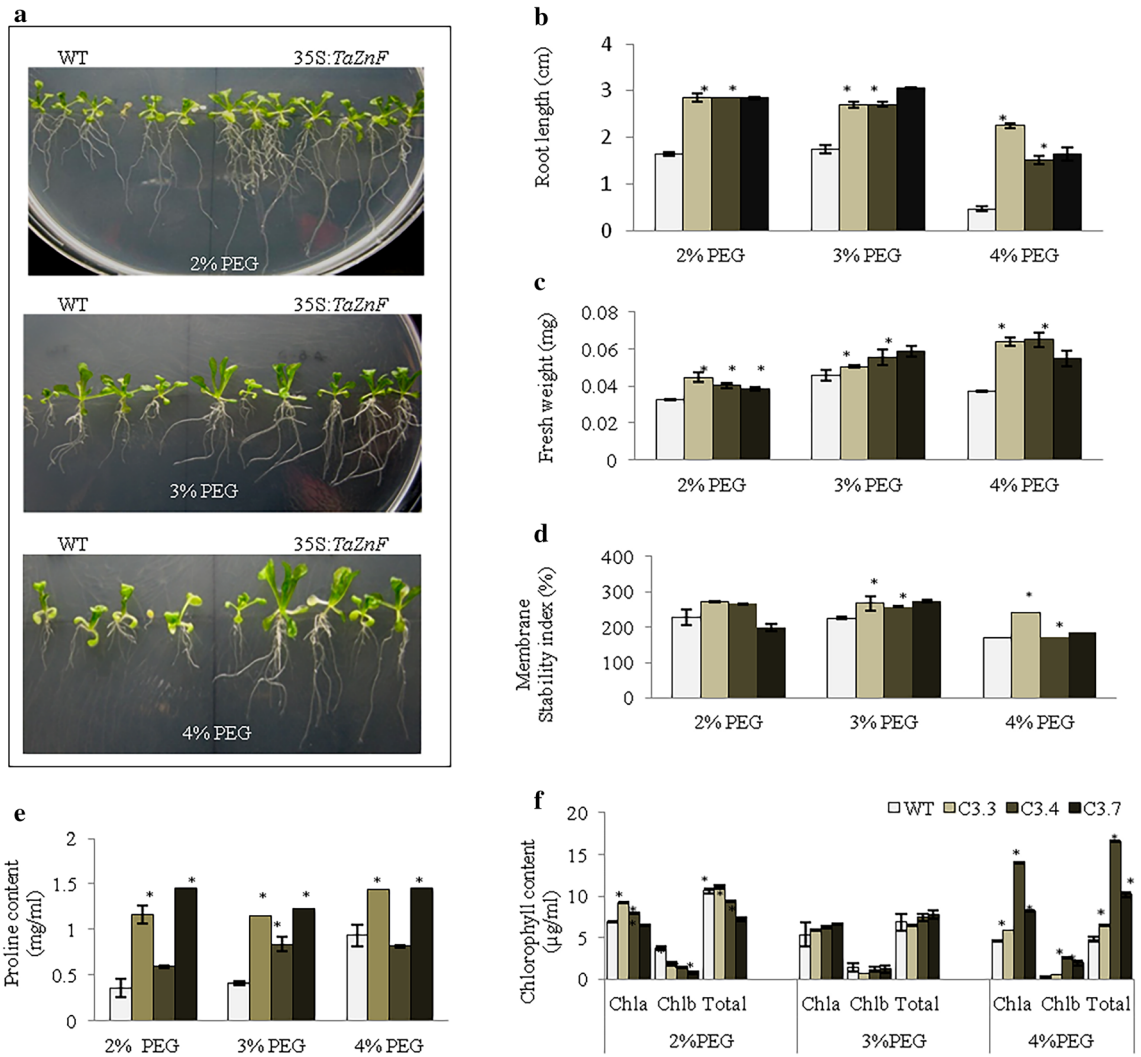

Fig. 2 *TaZnF* overexpressing *Arabidopsis* transgenics showed increased tolerance to dehydration stress. **a** Effect of dehydration stress on *Arabidopsis* transgenics and wild type (WT) plants was analyzed by growing seeds of both transgenics and WT on half MS media supplemented with various concentration of PEG (2%, 3% and 4%) and phenotype was observed after 20 days, **b** root length, **c** fresh weight, **d** membrane stability index, **e** proline content and **f** chlorophyll content. Asterisk* represent the t-test, *P* value of ≤ 0.05. Experiment represents the average value of three biological replicates

conditions as evident from their higher membrane stability, increased proline and chlorophyll content; an indicator for improved tolerance mechanism. Similar observation was made when transgenics were grown on half MS media supplemented with different concentration of mannitol (100 mM, 200 mM and 300 mM). The transgenics performed well in terms of their increased root length, more lateral branching, and higher fresh weight; and were found to be healthier in comparison to the wild type (Fig. S1a–c). They also showed higher accumulation of proline (Fig. S1d). Thus, these studies clearly indicate the possible role of *TaZnF* under dehydration stress. Since transgenics were found to perform better under dehydration stress, they were further analyzed for their overall growth and survival under drought stress. Both wild type and transgenic lines were subjected to drought stress for 14 days (refer to material and method section). The overexpression transgenics were found to be more tolerant as indicated by their survival rate (13% for C3.3, 13.33% for C3.4 and 11.67% C3.7), robust and healthier growth while the wild type plants perished under similar conditions (Fig. S3). Even after rewatering, the wild type did not recover while transgenics recovered well and showed profuse and healthier growth (Fig. 3). Further assays were done to examine the effect of overexpression on overall yield of the transgenic plants. For this one month old seedlings of both transgenics and wild type were subjected to drought stress for one month as mentioned in material and method sections. The transgenics were found to grow well and showed early bolting and flowering. The siliques of transgenics

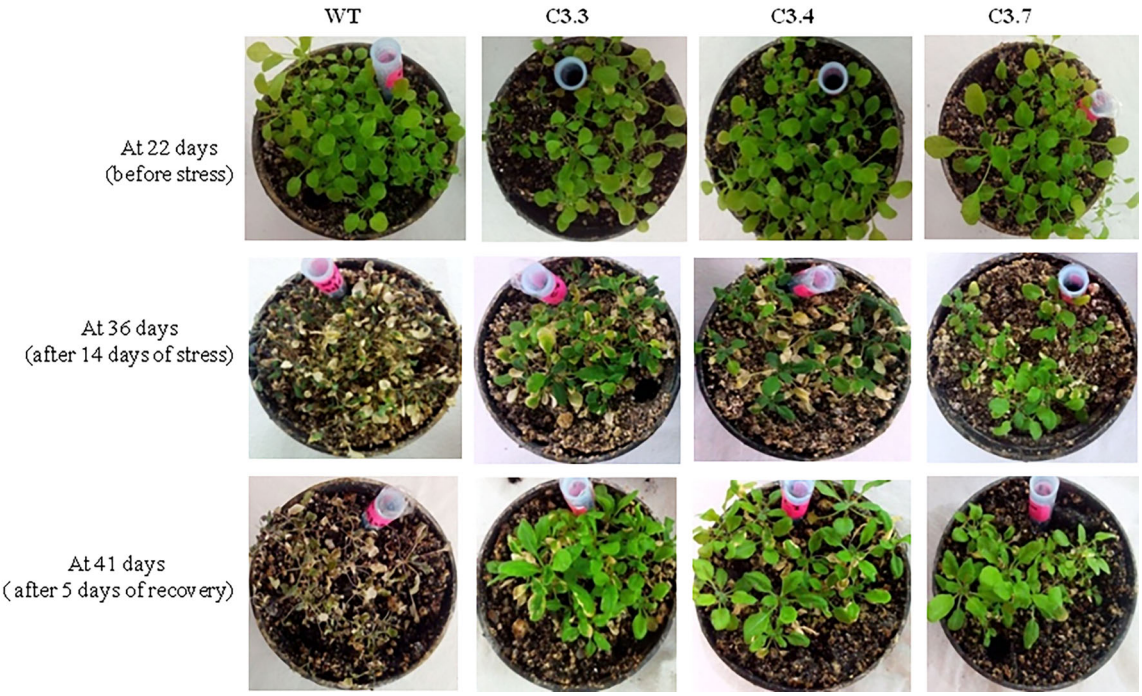

Fig. 3 *TaZnF* overexpressing *Arabidopsis* transgenics showed increased tolerance to drought stress. Effect of drought stress was analyzed by subjecting 22-day-old seedlings of both wild type (WT) and transgenics to drought treatment for 14 days and followed by recovery i.e. rewatering the plants for 5 days

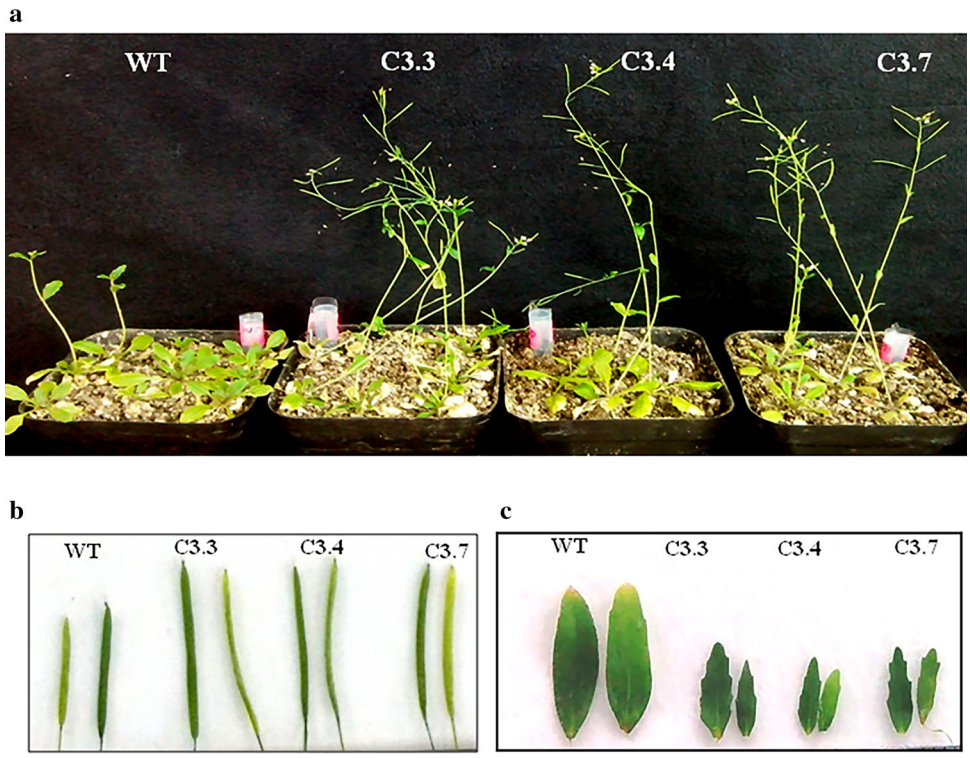

Fig. 4 *TaZnF* overexpressing *Arabidopsis* transgenics showed increased tolerance to drought stress. Effect of drought stress on one month old seedlings of transgenics and wild (WT) plants subjected to drought stress for one month. **a** Phenotype of wild type and transgenics observed after stress treatment, **b** size of siliques and **c** leaf morphology under drought stress

were larger in size and more in number; thus eventually leading to higher yield (Fig. 4a, b, Table 2). The leaves of transgenics were also found to be smaller in size, thus indicating the mobilization of carbon resource for seed setting, which further correlates with the early flowering and seed formation phenotype of transgenics (Fig. 4c).

Table 1 Representation of kind of stress treatment, plant stage, level of stress and parameters analysed

Plant stage	Kind of stress	Level of stress	Parameters measured
10-day-old seedling of PBW343	Salt (250 mM of NaCl)	0, 1, 2, 4, 6 and 8 h	Expression analysis of *TaZnF* (Fig. 1a, b)
10-day-old seedling of PBW343	Air dehydration	0, 1, 2, 4, 6 and 8 h	Expression analysis of *TaZnF* (Fig. 1a, b)
Seeds of both wild type and transgenics	½ MS media supplemented with 150 mM of NaCl.	48 h, 72 h and 96 h	Germination rate (after 48 h, 72 h and 96 h), root length and fresh weight (Fig S2 and Table 3)
Two weeks old seedlings of both wild type and transgenics	Gradual salt stress	200 mM NaCl for 5 days followed by 300 mM NaCl for next 16 days	Rosette diameter, leaf length, leaf breadth, fresh weight, membrane stability, proline content, and chlorophyll content (Fig. 5 and Table 3) and expression analysis of salt stress related marker genes (Fig. 6)
Seeds of both wild type and transgenics	Dehydration stress	½ MS media supplemented with different concentration of PEG (2%, 3% and 4%)	fresh weight, root length, proline content, chlorophyll content and membrane stability (Fig. 2)
Seeds of both wild type and transgenics	Dehydration stress	½ MS media supplemented with different concentration of mannitol (100 mM, 200 mM and 300 mM)	fresh weight, root length and proline content (Fig. S1)
22-day-old seedlings of both wild type and *Arabidopsis* transgenics grown in soilrite	Drought stress	Drought stress by not watering the plants for 14 days and then followed by re-watering for next 5 days	Phenotype regarding the survival and growth (Fig. 3 and Fig. S3)
One month old seedlings of both wild and *Arabidopsis* transgenics	Drought stress	Drought stress by not watering the plants for one month	Plant height, leaf length, leaf breadth, silique length, number of siliques per plant, seed yield per plant, proline and chlorophyll content (Fig. 4 and Table 2) and expression analysis of salt stress related marker genes (Fig. 6)

This is one of the most common mechanisms of the plants to escape and overcome stress conditions. The transgenics also showed higher proline and chlorophyll content (Table 2). Thus transgenics were found to be more tolerant under severe drought stress (one month) in comparison to wild type plants. These observations correlate with the function of other zinc finger proteins like *BrRZFP1* whose overexpression conferred increased tolerance to *Nicotiana tabacum* transgenics under dehydration stress (Jung et al. 2013). Similar observation was reported for tobacco transgenics overexpressing *AdZFP1* gene (tolerance to drought stress) and *DST* gene (increased tolerance in rice in response to drought and salinity stress) (Yang et al. 2008; Huang et al. 2009). In another analysis by Ben Saad et al. (2012) the expression of *AlSAP* in rice cv. Nipponbare, was found to enhance the tolerance to drought and salt stress. Further the expression of *AlSAP* did not show any loss in yield and allowed seed production even after exposure to severe drought stress at the vegetative stage. Collectively, these observations show that overexpression of *TaZnF* in *Arabidopsis* enhances the tolerance of transgenics under drought stress and thus it can be said that *TaZnF* functions as a positive regulator of drought stress response.

Table 2 Morphological and physiological parameters of *TaZnF* overexpressing *Arabidopsis* transgenic plants subjected to drought stress for one month

Parameters	WT	C3.3	C3.4	C3.7
Silique length (cm)	1.10 ± 0.05	1.60 ± 0.05*	1.70 ± 0.08*	1.80 ± 0.03*
No of siliques per plant	3.00 ± 0.50	13.00 ± 1.50*	12.00 ± 1.20*	13.0 ± 1.40*
Seed yield (mg/plant)	0.91 ± 0.13	3.10 ± 0.23	2.19 ± 0.21	2.19 ± 0.21
Plant height (cm)	5.70 ± 0.68	16.50 ± 0.17*	9.80 ± 1.49*	11.50 ± 1.44*
Leaf length (cm)	0.50 ± 0.05	0.30 ± 0.03	0.30 ± 0.03	0.30 ± 0.03
Leaf breadth (cm)	0.30 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
Proline content (mg/ml)	0.28 ± 0.01	2.52 ± 0.11*	0.89 ± 0.04*	1.09 ± 0.20
Chlorophyll content (mg/ml)	1.75 ± 0.15	7.82 ± 0.05*	6.26 ± 0.14*	4.97 ± 0.04*

*Represent P value of ≤ 0.05; ± sign represent standard error

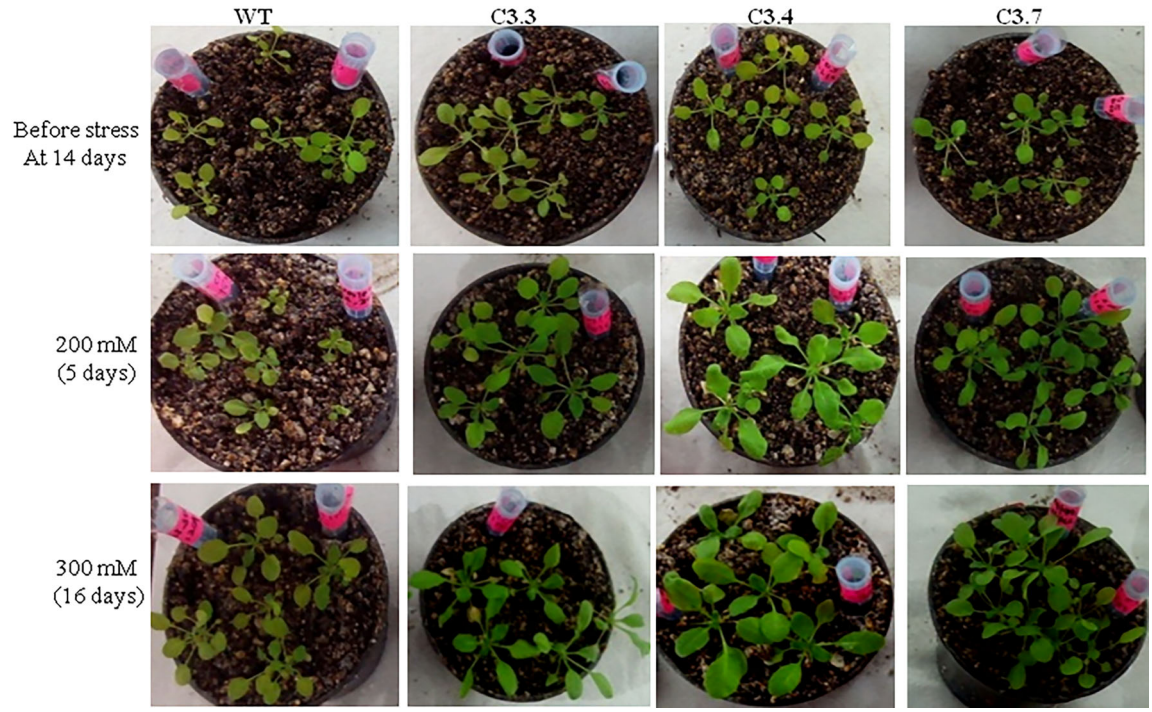

Fig. 5 *TaZnF* overexpressing *Arabidopsis* plants showed increased tolerance to salt stress. Effect of salt stress on 2 weeks old *TaZnF* overexpression *Arabidopsis* transgenic lines subjected to various concentration of salt stress i.e. 200 mM NaCl for 5 days followed by 300 mM NaCl for 16 days

Overexpression of *TaZnF* gene confers increased tolerance under salt stress

To find out the possible role of *TaZnF* in response to salt stress, seeds of both wild type and overexpression transgenics were grown on half MS supplemented with 150 mM of NaCl. The transgenics showed higher germination rate, longer root length and higher fresh weight (Table 3, Fig. S2a–b). Further studies were conducted to examine the overall growth and survival of transgenics under salt stress conditions. For this, transgenics and wild type plants were subjected to gradual salt stress treatment (refer to material and method section). The transgenics displayed profuse vegetative growth with larger leaves and rosette size in comparison to wild type plants which resulted in increased fresh weight of transgenics (Fig. 5, Table 3). According to previous studies, under salt stress plants collect a large number of compatible solutes like proline, polyols, trehalsose and betaine in the cytosol (Rhodes et al. 2002). Therefore, the effect of salt stress on proline accumulation was analysed and the transgenic plants were found to retain higher proline content (Table 3). These transgenics also showed higher membrane stability and more chlorophyll level than wild type under stress conditions (Table 3). These observations correspond with the previous study by Jung et al. (2013) where *BrRZFP1* overexpressing lines of tobacco have been reported to confer increased tolerance to salt stress as noted by their better growth with respect to their fresh weight, shoot and root length. Further, these transgenics also showed high retention of chlorophyll levels. Moreover, the overexpression *TaZNF* resulted in increased tolerance of *Arabidopsis* transgenic under salt stress conditions (Ma et al. 2016). Similarly, constitutive expression of *TaSAP17-D* in *Arabidopsis* conferred enhanced tolerance to transgenics under salt stress (Xu et al. 2018). In addition, *SpRing* gene belonging to RING finger E3 ligase has been also reported to be involved in salt stress signalling process in *Solanum piminellifolium.* The *Arabidopsis* transgenic overexpressing *SpRing* gene displayed increased tolerance to salt stress during germination and early stages of seed development (Qi et al. 2016). Transgenic tobacco expressing *AlSAP* gene also showed an improved tolerance to salinity and drought stress (Ben Saad et al. 2010). Overexpression of *ZAT6* also resulted in increased tolerance to rice transgenics under salt stress (Tang and Luo 2018). Thus, these observations together clearly indicate that overexpression of *TaZnF* confer increased tolerance to transgenics as they display better growth and robustness by retaining chlorophyll, accumulation of proline and increased membrane stability. Hence, these findings clearly indicate that *TaZnF* is an important determinant and plays a crucial role in several

Table 3 Morphological and physiological parameters of *TaZnF* overexpressing *Arabidopsis* transgenic plants subjected to salt stress

	Parameters \ Lines	WT	C3.3	C3.4	C3.7
Seedling stage	Fresh Weight (mg)	0.02±0.001	0.04±0.002*	0.04±0.001*	0.04±0.002*
	Root Length (cm)	0.29±0.02	1.14±0.03*	0.83±0.04*	1.28±0.03*
	Germination (%)	49.38±0.47	83.53±0.61*	86.39±0.65*	88.22±0.24*
Mature stage	Rosette Diameter (cm)	2.90±0.06	5.00±0.12*	4.47±0.09*	4.80±0.15*
	Leaf Length (cm)	0.64±0.12	1.43±0.12*	4.30±0.09*	4.80±0.15*
	Leaf Breadth (cm)	0.47±0.04	0.93±0.07*	0.80±0.06*	0.80±0.06*
	Fresh Weight (mg)	0.03±0.02	0.07±0.04*	0.08±0.03	0.05±0.04*
	Membrane Stability (%)	0.57±0.12	0.60±0.02	0.72±0.07	0.64±0.03
	Proline Content (mg/ml)	0.27±0.01	2.52±0.01*	0.89±0.04*	1.09±0.20*
	Chlorophyll Content (mg/ml)	2.72±0.01	3.40±0.25*	3.09±0.08	3.44±0.03*

* Represent P-value of ≤ 0.05; ±sign represent standard error.

stress-related processes and its overexpression can increase the tolerance or resistance of transgenic plants to salt stress.

Expression profiling of stress marker genes

To get an insight into the fact as to how the overexpression is leading to enhanced tolerance to drought and salt stress, expression profiling of various drought and salt stress marker genes were analysed in both wild and transgenic plants. The expression of these marker genes like *RD26, RD20, RD29A, RFD29B, DREB1A, DREB2A, ERD6, ATF1, SOS1, SOS2* and *SOS3* were found to be up-regulated in transgenics in comparison to wild type under stress conditions (Fig. 6). The C3.4 transgenic line showed higher level of expression in comparison to other two transgenic lines. The expression level of *RD29A, DREB1A* and *DREB2A* was found to be significantly higher (> 100 folds) in transgenics in comparison to the wild type under drought stress. The expression level of salt stress marker genes like *ATF1*, *SOS1*, *SOS2* and *SOS3* was also found to be higher (> 2 fold) in transgenics than the wild type under salt stress. Thus, the increased expression level of stress related genes closely correlates with the tolerant phenotype displayed by *TaZnF* overexpression transgenic plants under drought and salinity stress. Together these results demonstrate that *TaZnF* acts as a positive regulator of drought and salt stress via regulation of stress responsive genes in transgenic plants. These observations correspond with the previous report where the expression of salt stress related marker genes was found to be significantly high in *Arabidopsis* transgenics showing constitutive expression of *TaSAP17-D* in comparison to the wild type plants (Xu et al. 2018). Another report by Ben Saad et al. (2010) has shown that in tobacco transgenics expressing the *AlSAP* - gene, the expression level of stress related marker genes was higher than wild type plants. Similarly rice transgenics expressing *AlSAP* also showed higher transcript level of stress-related genes than wild type plants under both stress and control conditions (Ben Saad et al. 2012). Thus together these studies suggests that *TaZnF* is effective in providing tolerance upon overexpression and may be suitable for engineering in crop plants for corroborating

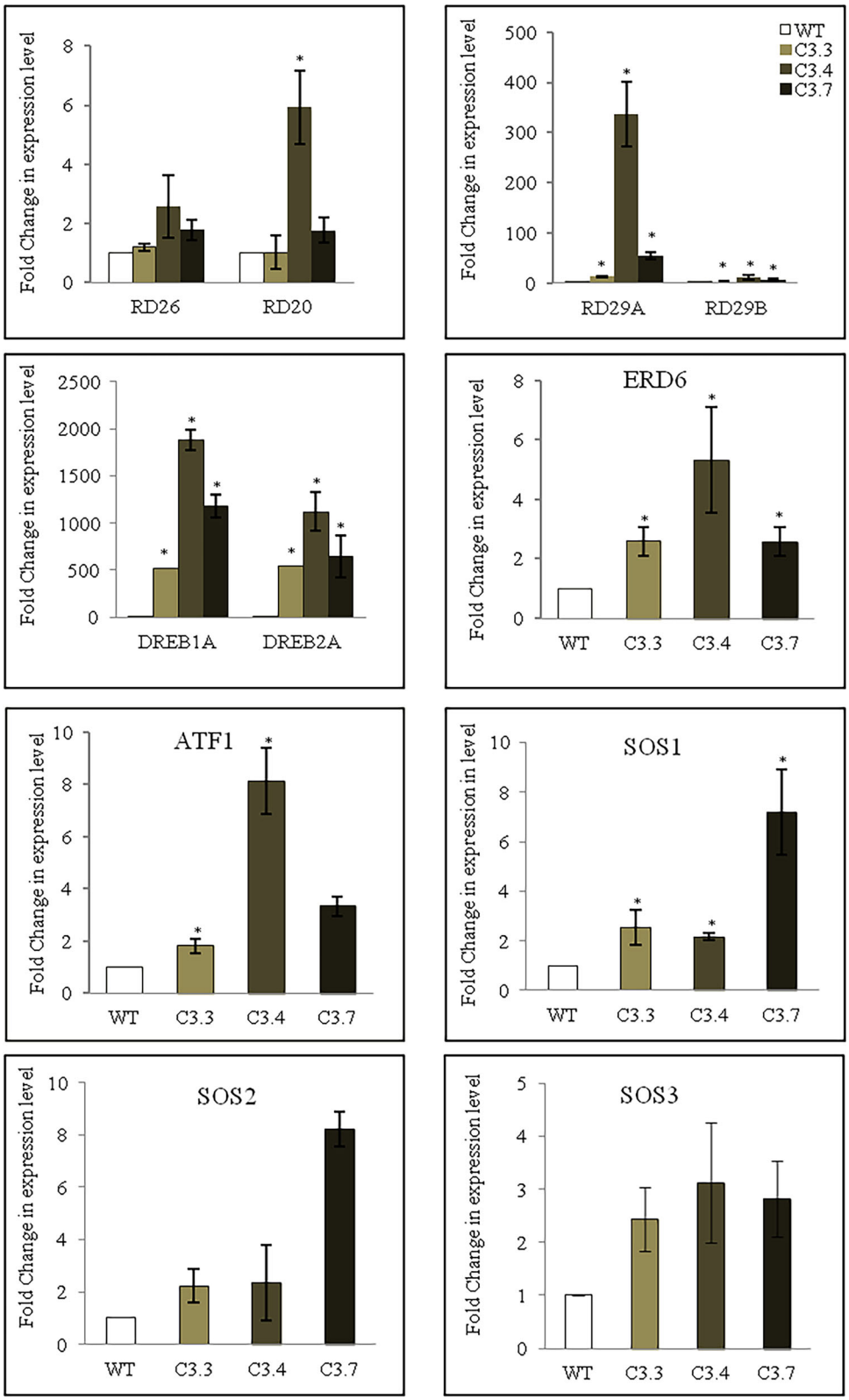

Fig. 6 Expression profiling of marker genes in wild type and transgenic lines of *Arabidopsis* plants overexpressing *TaZnF*. The expression level of wild type (WT) was normalized as 1.0 and the result shown are the means ± SDs of at least three independent experiments

suboptimal growth conditions and overcoming abiotic stresses.

Conclusions

To summarize *TaZnF* acts as a positive regulator for both drought and salt stress response. The overexpression of *TaZnF* resulted in increased tolerance to both drought and salt stress by regulating the expression of various stress related genes. Thus, these results indicate that *TaZnF* transcription factor is an important determinant of stress response in plants and changes in its expression level in plant might increase the tolerance level to various abiotic stresses. This further indicates that this gene can be used in genetic engineering for improving abiotic stress tolerance in crop plants.

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Author contribution PA and PK planned the experiment. PA wrote the manuscript. PA and PK read and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors state that they do not bear any conflict of interest.

Ethical approval This paper does not include any experiments with human participants or animals conducted by any of the authors.

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