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Original article

Effect of *AtPAP17* and *AtPAP26* genes overexpression on yield and yield components under salt stress in *Arabidopsis thaliana* plant

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Extended abstract

Introduction

Seed-yield as an important and quantitative trait for grain crops is determined by yield components, although it could also be adversely influenced by genotype and environment. Salinity limits seed yield via interfering with major physiological functions, disrupting ion homeostasis and diminishing nutrient uptake (such as phosphorus) in plant cells. Phosphorus plays an important role in photosynthesis, respiration, and regulation of a number of enzymes as well as signaling pathways. Due to the vital roles of phosphorus in cells, plant growth and productivity are frequently limited by low phosphorus availability. One of the adaptive changes of plants under phosphate deficient condition is the increase in phosphatase activity which is one of the primary plant responses to Pi releasing and recycling from both internal and external resources. Purple acid phosphatases (PAPs) are a group of APases that catalyze the hydrolysis of a wide range of phosphate esters and anhydrides in plants. The ultimate aim of salinity tolerance research is to increase the ability of plants to maintain growth and productivity in saline soils through the identification genes associated with responding to salt stress. Our current knowledge making *AtPAP17* and *AtPAP26* genes promising candidate for biotechnological strategies to improve Pi acquisition and utilization, and enhance yield components under NaCl stress condition.

Materials and methods

The *Arabidopsis thaliana* seeds, ecotype Columbia-0 (Col-0), atpap17 and atpap26 homozygous T-DNA insertion mutant lines (Mu17 and Mu26), double mutant of atpap17/26 (DM), AtPAP17 and AtPAP26 overexpressing lines (OE17 and OE26) were used. After seeds stratified at 4°C for 48 h, the plants were cultivated (1peat moss: 1perlite: 1cocopeat) in growth chambers with a 16 h light (1000 Lux), 8 h dark photoperiod at 25°C. Plants grown on this condition fertilized 48 hourly by subirrigation with similar Hoagland's solution containing 1.25 mM KH_2PO_4 for 28 days. Subsequently, the seedlings were subjected to salt stress by applying 50, 100, and 150 mM NaCl with the same Hoagland's solution containing 1.25 mM KH_2PO_4 for 16 days (long-term). The control plants were grown without addition of NaCl. Trend of flowering (for eight days after salt stress), pods number per plant, seeds number per

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each pod, and l000-seed weight obtained during the salt stress period. Finally, the total seed yield (total seed yield obtained from pods N. per plant × seed N. per each pod × l000-seed weight) was also calculated for all plants. Total phosphorus (TP) and free phosphorus inorganic (Pi) contents were also measured. There were three replications (with 15 plants on each replication) of each treatment. Least significant differences were used for means separation at the 0.01 probability levels.

Results and discussion

Results from the study revealed that the yield-related parameters and total yield as well as PT and Pi contents were gradually decreased in the genotypes with increase salt stress to 100 and 150 mM NaCl. However, no similar amount of decrease was observed among them under same growth conditions. Results showed that increase of NaCl concentration was associated with decreases in phosphorus accumulation in plants, and alternatively, phosphorus deficiency stress in plants caused to decline in the seed yield. The PT content of OE17 and OE26 were significantly higher than that in WT, although Pi content of OE17 was no-significantly higher, and Pi content of OE26 was significantly higher in compared with WT at 150 mM NaCl. These findings showed that AtPAP26 beyond AtPAP17 plays functional role in internal Pi-recycling or increasing the availability of Pi for plant by releasing Pi from external organophosphates when seedlings were deprived of the Pi supply.

DM plants did not have any pod at 150 mM NaCl, the DM plants (at both 100 and 150 mM NaCl) and Mu26 (at 150 mM NaCl) lacked any seed per pod, and also the number of seed per pod of Mu17 were significantly less than that of WT at 150 mM NaCl. In addition to these genotypes that did not have any seed, and subsequently no one-thousand-seed weight, the seeds of Mu17 showed lack viability, with increasing level of salt concentration and period of salt stress. Thus, Mu17 and Mu26 (at 150 mM NaCl), and DM (at both 100 and 150 mM NaCl) genotypes could not obtain total seed yield; However, OE17 and OE26 produced the highest total seed yield, under both 100 and 150 mM NaCl.

These results indicated that the plants responded to salinity depending on severity, duration of the stress and potential of them. Our results clearly demonstrated that overexpression of *AtPAP17* and *AtPAP26* genes is an effective approach to improve P acquisition. In addition, since AtPAP17 and AtPAP26 have both acid phosphatase and alkaline-peroxidase activity, they could be involved in phosphate scavenging and recycling as well as the metabolism of reactive oxygen species. These results could suggest that the physiological roles of AtPAP17 and AtPAP26 might be related to the adaptation of Arabidopsis to NaCl stress, possibly through its involvement in reactive oxygen species forming, scavenging and stress-responding signal transduction pathways.

Conclusions

It was clear that enhancing yield production was associated with Pi homeostasis in plants, and homeostasis of Pi for yield enhancement was related with the potential of the genotypes to recycle and scavenge Pi from intracellular and extracellular, and translocate Pi, under salt stress. Overall, the results suggest that *AtPAP17* and *AtPAP26* genes to supply of homeostasis of Pi could be used for the increase the ability to maintain of yield, under salt stress in Arabidopsis plants. Hence, the study of the positive effect of two AtPAP17 and AtPAP26 phosphatases on seed yield and seed yield components will be useful in generating of salt-tolerant crops.

Keywords*:* Mutant plants, Purple acid phosphatases, Seed yield improvement, Salt tolerance

Table 1. Analysis of variance of yield and yield components on *Arabidopsis thaliana* genotypes subjected under NaCl stress

		Mean Square					
Source of Variation	**Df**	**Slope of regression line**	**Flowering (%)**	**No. of pod plant $^{-1}$**	**No. of seed pod $^{-1}$**	**1000 Seed weight**	**Total seed yield**
Genotype	5	36.96**	1318.58**	684.12**	10.39**	167.02**	6.98**
Stress	3	37.38**	2023.17**	1529.21**	36.23**	391.95**	45.66**
Genotype×Stress	15	17.76*	498.51**	159.05**	4.38**	26.21**	1.00**
Error	48	8.56	169.01	4.67	1.31	0.84	0.38
CV (%)		*24.80*	*18.65*	*21.96*	*8.46*	*7.90*	*28.53*

* and **: Significant at the 5 and 1% level of probability, respectively

Table 2. Means (± SE) comparison of yield and yield components on *Arabidopsis thaliana* genotypes subjected to NaCl stress

Co. NaCl	**Geno-type**	**Slope of regression line**	**Flowering**	**No. of pod plant $^{-1}$**	**No. of seed pod $^{-1}$**	**1000 Seed weight (mg)**	**Total seed yield (mg plant^{-1})**
0mM	**WT**	11.24± 0.69^{ab}	65.04±5.1^{b}	6.44±1.3^{a}	36.00±0.6^{b}	17.00±0.2^{b}	3.92±0.8^{ab}
	Mu17	4.89± 0.66 ^{c}	25.95±1.0^{c}	2.66±0.2^{b}	35.00±1.7^{b}	15.73±0.5^{c}	3.47±0.1^{b}
	Mu26	9.86± 1.50 ^{b}	61.62±3.6^{b}	6.51±0.3^{a}	34.67±1.2^{b}	16.40±0.1^{b}	3.70±0.2^{ab}
	DM	11.69± 1.35^{ab}	71.11± 4.4^{b}	8.78±0.9^{a}	36.33±0.3^{b}	15.20±0.1^{c}	4.86±0.5^{ab}
	OE17	15.38± 1.1b ^{a}	94.44± 5.5^{a}	8.42±1.2^{a}	40.67±1.2^{a}	18.07±0.4^{a}	5.27±0.6^{a}
	OE26	13.29 ± 2.16^{ab}	76.67± 6.7^{b}	7.89±0.4^{a}	33.33±0.7^{b}	18.07±0.2^{a}	4.77±0.3^{ab}
50mM	**WT**	11.72± 0.72^{b}	51.19± 1.9^{c}	6.70±1.1^{a}	32.00±1.5^{b}	15.67±0.2^{b}	3.32±0.4^{a}
	Mu17	12.42± 0.40^{b}	74.60±12.9^{ab}	3.47±0.8^{b}	17.67±1.4^{c}	11.93±0.8^{c}	0.71±0.1^{b}
	Mu26	13.21± 1.80 ^{b}	75.84± 7.3^{ab}	5.71±0.6^{ab}	28.00±1.5^{b}	13.00±0.3^{c}	2.10±0.3^{ab}
	DM	13.70± 0.89^{ab}	81.57± 4.2^{b}	6.01±0·8^{ab}	21.00±1.0^{c}	9.00±0.2^{d}	1.12±0.1^{b}
	OE17	15.08± 0.49 ^{a}	90.58± 1.2^{a}	5.81±1.1^{ab}	36.67±1.2^{a}	17.13±0.8^{a}	3.69±0.8^{a}
	OE26	16.68± 1.46 ^{a}	88.64± 7.3^{a}	6.59±0.8^{a}	38.33±0.7^{a}	15.13±0.5^{b}	2.90±0.8^{a}
100mM	**WT**	11. 75± 0.18^{b}	81.82± 2.0^{ab}	4.27±0.5^{b}	28.33±3.5^{b}	12.06±1.8^{b}	1.45±0.3^{c}
	Mu17	12.39± 1.46 ^{b}	71.14± 5.3^{b}	4.35±0.3^{b}	22.33±1.4^{c}	9.733±0.3^{b}	0.94±0.1^{d}
	Mu26	11.79± 0.92 ^{b}	71.15± 4.5^{b}	6.75±0.4^{a}	25.67±0.3^{bc}	11.40±0.4^{b}	1.73±0.0^{bc}
	DM	11.56± 0.90 ^{b}	70.85± 7.9^{b}	5.01±0.6^{b}	0.00±0.0^{d}	0.00±0.0^{c}	0.00±0.0^{e}
	OE17	15.27± 0.64 ^{a}	90.11± 5.0^{a}	4.12±0.2^{b}	26.67±0.3^{bc}	15.26±0.2^{a}	2.14±0.1^{a}
	OE26	11.81± 0.33 ^{b}	69.80± 1.6^{b}	5.33±0.3^{b}	34.33±0.7^{a}	12.00±0.4^{b}	2.19±0.1^{a}
150mM	**WT**	8.73± 1.51^{ab}	54.44±10.9^{a}	3.23±0.4^{b}	21.33±2.1^{b}	9.60±0.3^{c}	0.67±0.1^{c}
	Mu17	8.05± 2.39^{ab}	47.59± 5.5^{ab}	3.05±0.3^{b}	10.00±0.0^{c}	0.00±0.0^{d}	0.00±0.0^{d}
	Mu26	9.98± 0.82 ^{a}	60.32± 5.^{2a}	4.50±0.4^{a}	0.00±0.0^{d}	0.00±0.0^{d}	0.00±0.0^{d}
	DM	6.07± 0.80 ^{c}	33.12±12.0^{b}	0.00±0.0 ^{c}	0.00±0.0^{d}	0.00±0.0^{d}	0.00±0.0^{d}
	OE17	10.12± 0.29 ^{a}	64.50± 4.5^{a}	3.90±0.2^{b}	31.67±1.2^{a}	14.33±1.0^{a}	1.88±0.2^{a}
	OE26	10.74± 0.35 ^{a}	65.48±3.0^{a}	3.22±0.2^{b}	22.67±0.3^{b}	11.53±0.6^{b}	1.27±0.1^{b}

Mean values for each level of NaCl concentrations in a column with different letters are significantly different at $P < 0.01$.

Table 3. Analysis of variance of the yield and yield components on *Arabidopsis thaliana* genotypes subjected under NaCl stress

Source of Variation	df	Mean Square	
		TP Content	Pi Content
Genotype	5	415.34**	41.57**
Stress	3	687.02**	101.33**
Genotype×Stress	15	31.80**	3.15**
Error	48	1.63	1.00
C.V. (%)		12.55	7.15**

**: Significant at the 1% level of probability

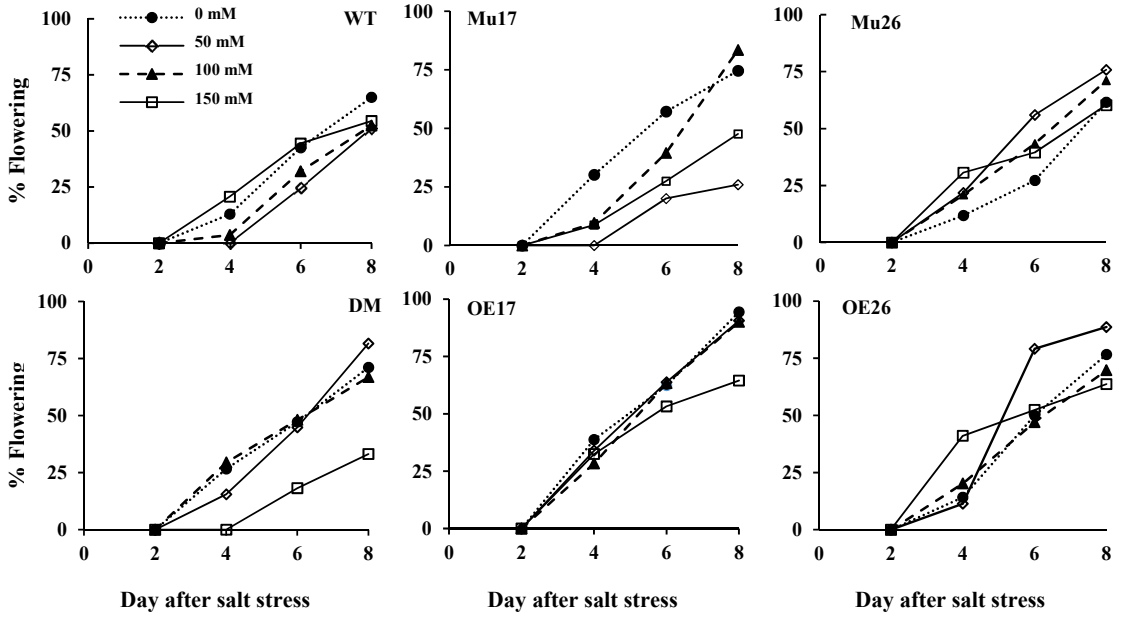

Fig. 1. Trend of flowering on *Arabidopsis thaliana* genotypes subjected to 0, 50, 100 and 150 mM NaCl. Wild type (WT), overexpress (OE), single mutant (Mu), and double mutant (DM) genotypes for *AtPAP17* and *AtPAP26* genes. After 28 days growing at normal condition, the plants were subjected to salt stress by applying 50, 100 and 150 mM NaCl concentrations involving 1.25 mM KH_2PO_4. The control plants were grown without addition of NaCl. Flowering percentage were measured at 2, 4, 6, and 8 days after applying salt stress. There were three replications (with 15-20 plants on each replication) of each treatment.

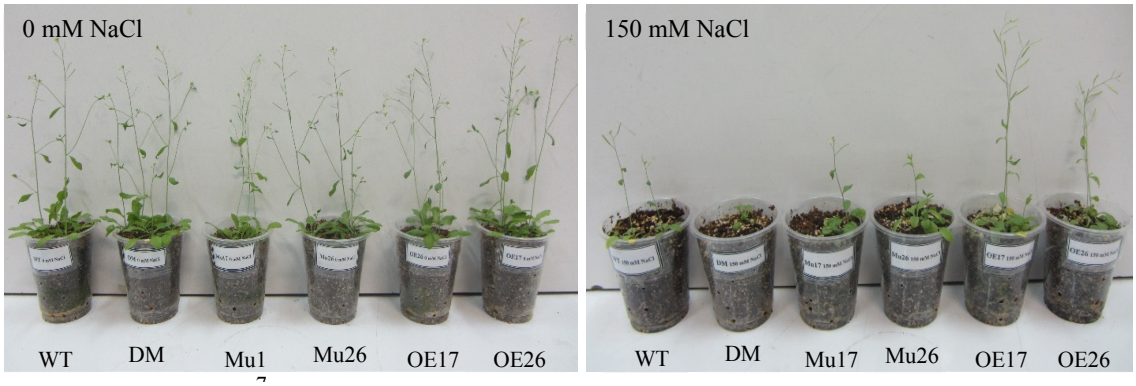

Fig. 2. The growth changes of the *Arabidopsis thaliana* genotypes during the reproductive stage at 0 and 150 mM NaCl. Wild type (WT), overexpress (OE), single mutant (Mu), and double mutant (DM) genotypes for *AtPAP17* and *AtPAP26* genes.

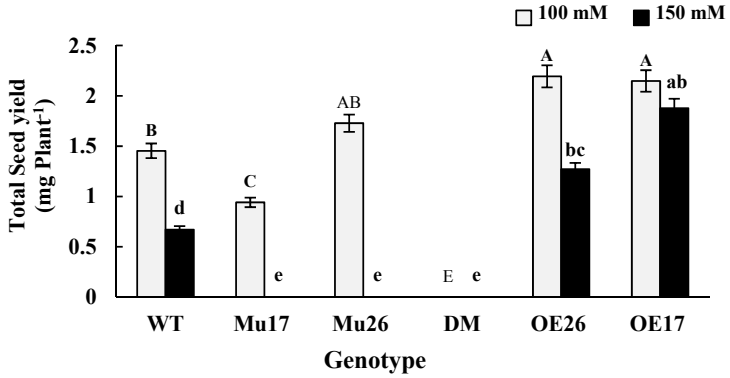

Fig. 3. The changes in total yield of overexpress and mutant *Arabidopsis thaliana* genotypes of *AtPAP17* and *AtPAP26* genes treated at 100 and 150 mM NaCl concentrations. Wild type (WT), overexpress (OE), single mutant (Mu), and double mutant (DM) genotypes for *AtPAP17* and *AtPAP26* genes. There were three replications (with 15-20 plants on each replication) of each treatment. The significant mean differences ($P < 0.01$) were separately shown at each level of salt stress with uppercase and lowercase.

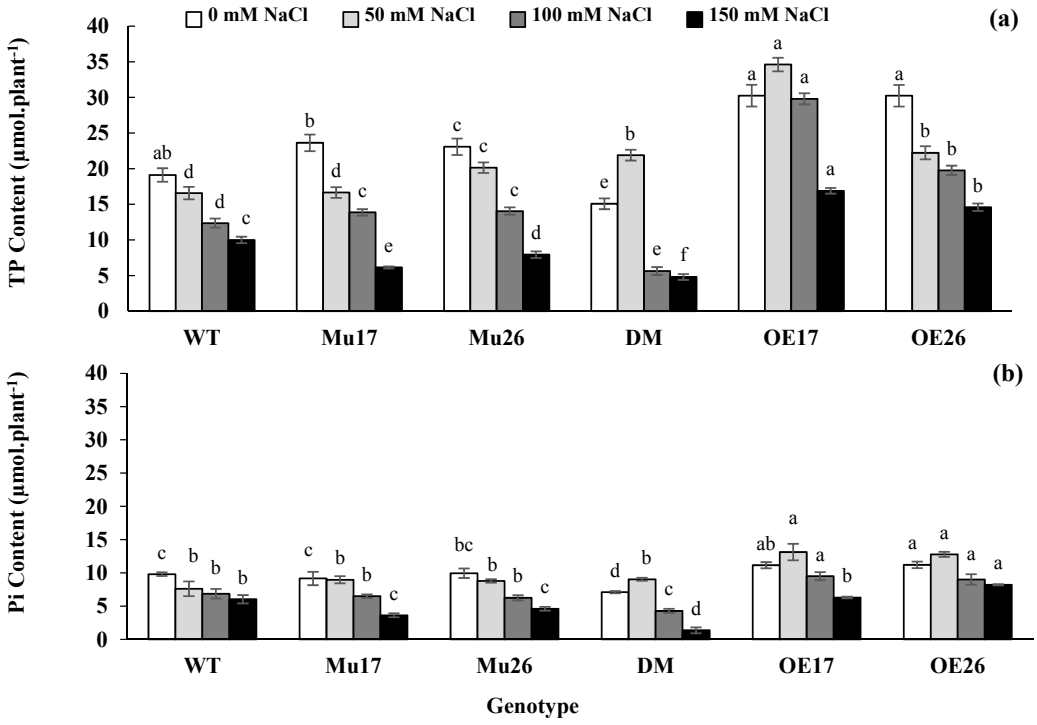

Fig. 4. The total and inorganic phosphate content in *Arabidopsis thaliana* genotypes subjected to the NaCl concentrations. Wild type (WT), overexpress (OE), single mutant (Mu), and double mutant (DM) genotypes for *AtPAP17* and *AtPAP26* genes. After 28 days growing at normal condition, the plants were subjected to salt stress by applying 50, 100 and 150 mM NaCl concentrations involving 1.25 mM KH_2PO_4. The control plants were grown without addition of NaCl. PT and Pi content were measured on 35-day-old plants. There were three replications (with 15-20 plants on each replication) of each treatment. The significant mean differences ($P \leq 0.01$) were shown with different letters.