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Competing interests

No competing interests have been declared.

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ORIGINAL RESEARCH PAPER

Putrescine catabolism via DAO contributes to proline and GABA accumulation in roots of lupine seedlings growing under salt stress

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* Corresponding author. Email: legocka@amu.edu.pl**Abstract**

The levels of polyamines (PAs), proline (Pro), and γ -aminobutyric acid (GABA) as well as the activity of diamine oxidase (DAO; EC 1.4.3.6) were studied in the roots of 2-day-old lupine (*Lupinus luteus* L. 'Juno') seedlings treated with 200 mM NaCl for 24 h. The effect of adding 1 mM aminoguanidine (AG), an inhibitor of DAO activity, was also analyzed. It was found that in roots of lupine seedlings growing under salt stress, a negative correlation between Pro accumulation and putrescine (Put) content takes place. Pro level increased in roots by about 160% and, at the same time, Put content decreased by about 60%, as a result of ca. twofold increase of DAO activity. The AG added to the seedlings almost totally inhibited the activity of DAO, increased Put accumulation to control level, decreased Pro content by about 25%, and reduced GABA level by about 22%. Addition of 50 mM GABA to the lupine seedlings growing in the presence of AG and NaCl restored Pro content in roots to its level in NaCl-treated plants. In this research, the clear correlation between Put degradation and GABA and Pro accumulation was shown for the first time in the roots of seedlings growing under salt stress. This could be considered as a short-term response of a plant to high salt concentration. Our findings indicate that during intensive Pro accumulation in roots induced by salt stress, the pool of this amino acid is indirectly supported by GABA production as a result of Put degradation.

Keywordsdiamine oxidase; lupine roots; polyamines; proline; salt stress; γ -aminobutyric acid**Introduction**

Salt stress is one of the major factors limiting crop production. It causes a wide variety of physiological and biochemical changes in plants. Polyamines (PAs), in combination with proline (Pro) and γ -aminobutyric (GABA), belong to the group of agents with almost universal involvement in a variety of stress responses [1–5]. Accumulation of Pro in stressed plants, up to 100 times to the normal level, has been a well-known fact for more than 40 years [6]. Pro accumulation during osmotic stress is mainly due to increased synthesis and reduced degradation, and it has been suggested to contribute to stress tolerance in many ways [1,3]. Apart from acting as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular structures (e.g., membranes and proteins), scavenging free radicals, and buffering cellular redox potential under stress conditions [3,7]. Pro itself may also serve as a nitrogen and carbon source needed in stress recovery [8,9].

Investigations involving a number of plant species have shown that changes in concentrations of PAs represent a common plant response to a variety of abiotic stresses. The function of PAs is presumed to be protective, as they act as free radical scavengers, maintain cellular pH balance, interact with macromolecules like DNA, RNA, proteins, especially membrane lipids and proteins, preventing these macromolecules and membranes from denaturation under stress [2,4]. The abundance of data concerning stress-dependent PAs accumulation is well known [4,10]. The accumulation of Put in the intact plants and leaves in response to the osmotic or salt stresses has been reported by many authors [2,11,12]. However, others have reported either decrease or no changes at all in the Put level or in the level of all three major PAs in response to salt stress [13–15].

The high level of GABA accumulated in response to different environmental stresses and GABA metabolism have different functions such as regulation of pH and osmotic pressure, bypass of tricarboxylic acid cycle, and C–N balance [5,16,17]. GABA is mainly produced by the irreversible reaction catalyzed by the cytosolic enzyme glutamate decarboxylase (GAD; EC 4.1.1.15) [16,18]. Besides GABA shunt pathway, GABA synthesis may occur via PAs (Put and Spd – spermidine) degradation [5,16,19]. It is suggested by Shelp et al. [19] that in alternative pathways to GABA synthesis, the primary metabolite is Put.

Interesting studies demonstrate that under salt stress Put catabolism via DAO contributes to Pro accumulation and provides about one third of the total GABA content [9,20–23]. It has been shown that in response to salt stress, DAO and polyamine oxidase (PAO; EC 1.5.3.11) activity is prompted in tomato leaves, soybean roots, and in rice roots and shoots [9,21,24].

In our study, we were interested in Put oxidation, because results of other authors suggest that Put catabolism plays a major role in the production of GABA in plants under stress. In soybean hypocotyl exposed to NaCl *in vitro*, Cu-containing amine oxidase (CuAO) activity was up to 77-fold higher than that of polyamine oxidase [25]. Oxidation of PAs (in apoplast) leads to accumulation of GABA through stimulation of CuAO activity under salt stress [21]. In the germinating fava bean under hypoxia-NaCl stress, polyamine degradation pathway, particularly Put, provided approximately one-third of GABA formation [23]. Direct evidence for the production of GABA from Put is provided by the experiments with radiolabeled putrescine exogenously supplied to the roots of maize [26] and *Limonium tataricum* [27], discs of tomato pericarp [28], and excised cotyledons of *Pinus radiata* [29]. The accumulation of radiolabeled GABA is suppressed by addition aminoguanidine (AG), a diamine oxidase inhibitor [27]. For that reason, we decided to analyze DAO activity and the effects of AG application on GABA formation in PA degradation pathway.

The combined study of stress-inducible metabolites such as PAs and Pro constitutes a useful tool to understand better the mechanisms of plant adaptation to the environment. This approach allows to investigate in the different models the relationship between Put catabolism and Pro accumulation. There is only limited information on the rates of PA degradation and Pro accumulation under salt stress. To date, in a few cases only, such as tomato leaf discs [9], *Fraxinus angustifolia* callus [20], and soybean leaves [21,22] subjected to salt stress, it was shown that polyamine degradation promoted a proline accumulation.

To our knowledge, there is no research on this correlation in the roots of plants growing under salt stress. The high Pro level was found in flowers and seeds, and the lowest in roots [1]. The apparent discrepancy in the roots of *Arabidopsis* between low Pro content and high levels of the transcripts and protein products of genes coding for pyrroline-5-carboxylate synthase (*P5CS*) and pyrroline-5-carboxylate reductase (*P5CR*) can be explained by Pro export via xylem to the shoot [30,31].

In our experiments, we used the roots of 2-day-old lupine seedlings (*Lupinus luteus* L. ‘Juno’) treated with 200 mM NaCl for 24 h. In this model, an export of Pro from roots to upper parts of the plant practically does not exist as the shoots are not yet developed. In the roots of lupine seedling, we analyzed free PA titer (Put, Spd, Spm – spermine), DAO activity, Pro and GABA levels. To check a link between Put catabolism and accumulation of Pro and GABA in the roots of seedlings growing in stress condition, an inhibitor of DAO activity AG was used. Additionally, the Pro level was examined in the roots of seedlings growing in the presence of NaCl, AG, and exogenous GABA.

Material and methods

Lupine seeds (*Lupinus luteus* L. 'Juno') were sterilized with 20% commercial bleaching solution for 10 min, rinsed and soaked in distilled water, and germinated on several layers of moist tissue paper in plastic boxes in the dark at 23°C. Two-day-old seedlings were incubated for 24 h in the dark in Hoagland solution (HS) (control) and in HS with 200 mM NaCl. Additionally to control was added separately AG, Put, and AG with Put. The analogical treatment was used in NaCl probes. To verify the participation of GABA in Pro accumulation in the roots of seedlings growing under salt stress, exogenously added GABA in 20 and 50 mM concentration was added to NaCl probes without or with AG.

HPLC polyamine analysis

Quantitative and qualitative analysis of polyamines was performed by HPLC method according to Marcé et al. [32] using the Varian 900-LC chromatograph (Agilent, Australia). Polyamines were dansylated and collected with toluene, then, after toluene evaporation, dissolved in 800 μ L acetonitrile. The sample (5–20 μ L) was applied to a column of Spherisorb 5 μ m ODS2 (4.6 \times 100 mm; Waters, USA) with guard column C18 (Supelguard Discovery 2 cm \times 4.0 mm, 5 μ m; Sigma-Aldrich, Germany); flow rate 1.5 mL min⁻¹. Gradient elution was performed with acetonitrile (solvent A) and water (solvent B): 0–4 min, 70% A; 4–5 min, 70–100% A; 5–9 min, 100% A; 9–10 min, 100–70% A; 10–15 min, 70% A. The flow of dansylated polyamines was monitored using a Varian's Prostar 363 fluorescence detector (Agilent) (excitation at 252 nm, emission at 500 nm). Retention times of the different polyamines were as follows: 1.8 min for diaminopropane, 2.2 min for Put, 3.35 min for diaminoheptane (internal reference), 5.46 min for Spd, and 6.63 min for Spm.

Proline extraction and determination

Pro was determined by the ninhydrine method according to Bates et al. [33]. Roots (250 mg) were extracted with 2.5 mL 3% 5-sulphosalicylic acid, centrifuged at 10 000 g and 1 mL of the supernatant was used for the assay. Absorbance of the organic layer (toluene) was measured at 520 nm with spectrophotometer. Calibration was made using L-Pro as standard.

Determination of DAO activity

Crude enzyme extract, obtained according to the protocol described by Su et al. [22], was used to determine DAO activity. Reaction solutions (3.0 mL) contained 2.5 mL 0.1 M sodium phosphate buffer (pH 6.5), 0.1 mL crude enzyme extracts, 0.1 mL peroxidase (250 U mL⁻¹), and 0.2 mL 4-aminoantipyrine / *N,N*-dimethylaniline. The reaction was initiated by the addition of 0.1 mL 20 mM Put. A 0.01 value of changes in the absorbance at 555 nm was regarded as one activity unit of the enzyme.

GABA extraction and determination

The total metabolite extraction from roots followed the procedure described earlier [34], with certain modifications. Briefly, 50 mg of frozen samples were suspended in 1.4 mL of 80% methanol. The mixtures were placed in microwave bath (Bendelin Electronics, Germany) for 20 min at room temperature. The suspensions were centrifuged at 11 000 g at room temperature. The supernatants were evaporated using vacuum concentrator (Eppendorf, Germany). The dried extract samples were dissolved in 50 μ L of 20 mg/mL methoxamine hydrochloride solution in pyridine, and the derivatization reaction was performed for 1.5 h followed by a 30 min reaction with 80 μ L of *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA). Both reactions were performed at 37°C.

Qualitative and quantitative analyses were performed using a TRACE 1300 series gas chromatograph (Thermo Scientific, USA) and a TSQ8000 mass spectrometer (Thermo Scientific) with Thermo Xcalibur software version 2.2. Gas chromatography was performed using a DB-5MS capillary column (30 m × 0.25 mm with a 0.25- μ m film thickness; J & W Scientific, USA). The injection was performed using programmed temperature vaporizer inlet, with temperature gradient from 40 to 250°C, the mass spectrophotometer transfer line was at 250°C, and the ion source was adjusted to 250°C. Pure helium was used as a carrier gas at a constant flow of 1.2 mL/min. The oven temperature was maintained at 70°C for 1 min, then ramped at 15°C/min to 300°C, and finally maintained at 300°C for 10 min. Mass spectra were recorded in the m/z range 50–850 with electron ionization (70 eV). Additional scan was set to specifically measure the transitions of selected ions fragmentation, namely fragmentation of 304 m/z to 174 m/z for γ -aminobutyric acid – GABA (retention time = 8.56 min) was traced. Three biological replicates of each treated sample were analyzed. GC-MS data were processed using NIST spectral database (qualitative identification) and further statistically elucidated for GABA content. All chromatograms were traced for 304 m/z → 174 m/z transition, specific for GABA fragmentation. Areas under the peaks were calculated for single reaction monitoring transitions and for each other signal in total ion current chromatogram for normalization to total ion current. All data were transferred to Excel software (Microsoft Corporation, USA) where data were normalized and

mean values together with standard deviations were calculated for GABA content in the samples.

The differences in the measured parameters were analyzed for statistical significance using one-way analysis of variance (ANOVA) and Tukey–Kramer multiple comparison test. Means were considered as significantly different at p value <0.01. The statistical analysis was performed using STATISTICA software (StatSoft, Inc., USA).

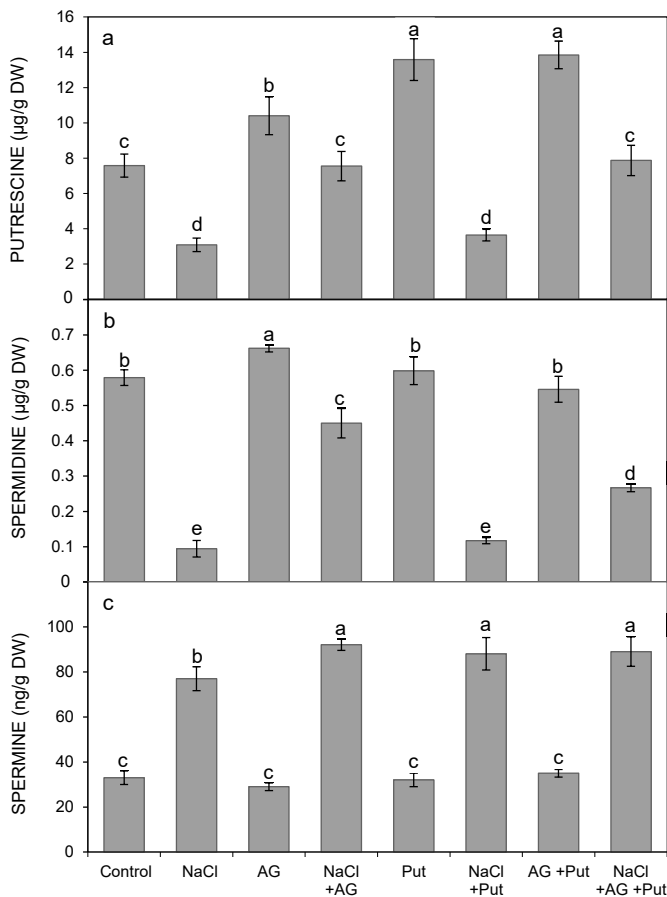


Fig. 1 The content of polyamines: putrescine (a), spermidine (b), spermine (c) in the roots of 3-day-old lupine seedlings incubated in HS (control) and/or HS with 200 mM NaCl in the absence or presence of 1 mM AG and 1 mM Put for 24 h in the dark. The values are the means of three replicates of measurements obtained in four independent experiments. The differences in the measured parameters were analyzed for statistical significance using one-way analysis of variance (ANOVA) and the Tukey–Kramer multiple comparison test ($n = 6$, $p < 0.01$). The same letter on the bars indicates that there were no significant differences between the means.

Results

Content of PAs in the roots of lupine seedlings growing under salt stress in the absence or presence of exogenously added Put and AG

Put was a dominating polyamine in the roots of lupine seedlings. The results showed that in salt-treated seedlings for 24 h, Put content decreased to 60% compared to the control (Fig. 1a). Application of AG to NaCl-treated plants for 24 h caused an increase in Put content almost to the control level (Fig. 1a). Addition of 1 mM exogenous Put to the seedlings growing under salt stress for 24 h resulted in an increase in Put level in roots by about 20% compared to the salt alone, and this tendency was observed when Put and AG were added together to the salt-treated plants (Fig. 1a). Exogenous Put added together with AG to the control seedlings for 24 h resulted in an increase in Put content in roots by about 80% compared to the control (Fig. 1a).

In the roots of lupine seedlings growing under salt stress for 24 h, the 85% decrease in Spd level was observed as compared to control (Fig. 1b). Addition of AG to the salt-treated seedlings caused 3.4-fold increase of Spd level in roots in comparison to the salt treatment alone (Fig. 1b). In the roots of seedlings growing under salt stress, a significant 140%

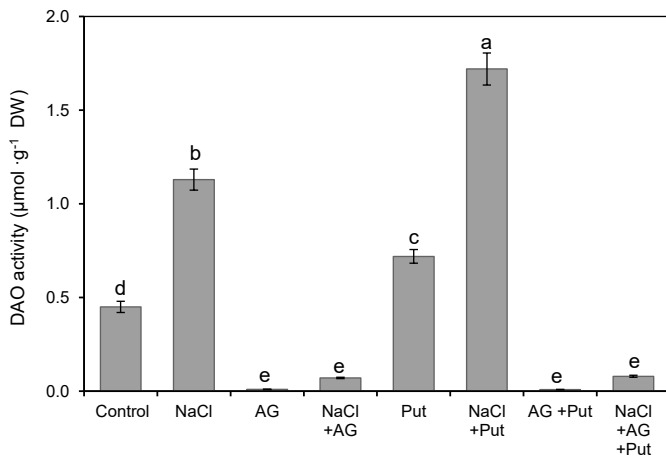


Fig. 2 DAO activity in the roots of 3-day-old lupine seedlings incubated in HS (control) and/or HS with 200 mM NaCl in the absence or presence of 1 mM AG and 1 mM Put for 24 h in the dark. The values are the means of three replicates of measurements obtained in four independent experiments. The differences in the measured parameters were analyzed for statistical significance using one-way analysis of variance (ANOVA) and the Tukey–Kramer multiple comparison test ($n = 9, p < 0.01$). The same letter on the bars indicates that there were no significant differences between the means.

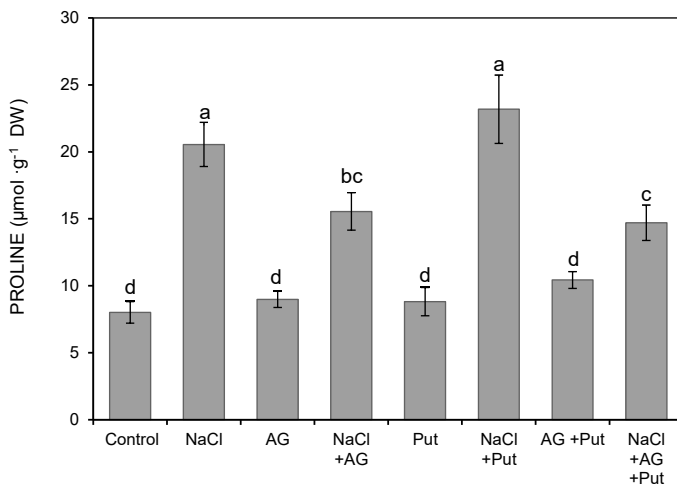


Fig. 3 The content of proline in the roots of 3-day-old lupine seedlings incubated in HS (control) and/or HS with 200 mM NaCl in the absence or presence of 1 mM AG and 1 mM Put for 24 h in the dark. The values are the means of three replicates of measurements obtained in four independent experiments. The differences in the measured parameters were analyzed for statistical significance using one-way analysis of variance (ANOVA) and the Tukey–Kramer multiple comparison test ($n = 12, p < 0.01$). The same letter on the bars indicates that there were no significant differences between the means.

increase in Spm content was noticed as compared to the control (Fig. 1c). Addition of Put and/or AG slightly increased the Spm level.

DAO activity in the roots of lupine seedlings growing under salt stress in the absence or presence of exogenously added Put and AG

In the roots of seedlings incubated in salt for 24 h, DAO activity increased 1.5 times in comparison to the control (Fig. 2). Exogenously added Put to salt-treated seedlings resulted in an increase in DAO activity by about 51% as compared to NaCl control (Fig. 2). Application of Put to the control seedlings raised DAO activity in roots by about 53%. AG applied to control seedlings or salt-stressed seedlings almost completely reduced DAO activity in roots (Fig. 2).

Proline level in the roots of lupine seedlings growing under salt stress in the absence or presence of exogenously added Put and AG

Parallel to the decrease in Put and Spd content in the roots of seedlings growing under salt stress, a significant increase in Pro content was detected. In the roots of seedlings growing for 24 h in the presence of NaCl, the level of this amino acid was about 160% higher as compared to the control (Fig. 3). When AG was supplied to NaCl solution, Pro content in roots decreased by about 25% in comparison to salt-treated seedlings (Fig. 3). Exogenously added Put to this variant had insignificant effect on Pro level (Fig. 3).

GABA level in the roots of lupine seedlings growing under salt stress in the absence or presence of exogenously added AG

In the roots of NaCl treated seedlings the level of GABA was threefold higher than that in control (Fig. 4b). When seedlings were incubated in NaCl together with AG, GABA level in roots decreased by about 22% in comparison to that observed in the case of treatment with NaCl alone (Fig. 4b). The roots of seedlings growing under salt stress were significantly shorter than in the control, and in the presence of AG in NaCl medium, roots were little longer than in NaCl only (Fig. 4a).

Proline level in the roots of lupine seedlings growing under salt stress in the absence or presence of exogenously added GABA

Addition of 20 or 50 mM GABA to the seedlings incubated for 24 h in NaCl did not change Pro level in roots. AG applied to NaCl-treated seedlings reduced Pro level in roots by about 26% as it was shown earlier in Fig. 3. Fifty mM GABA added to the

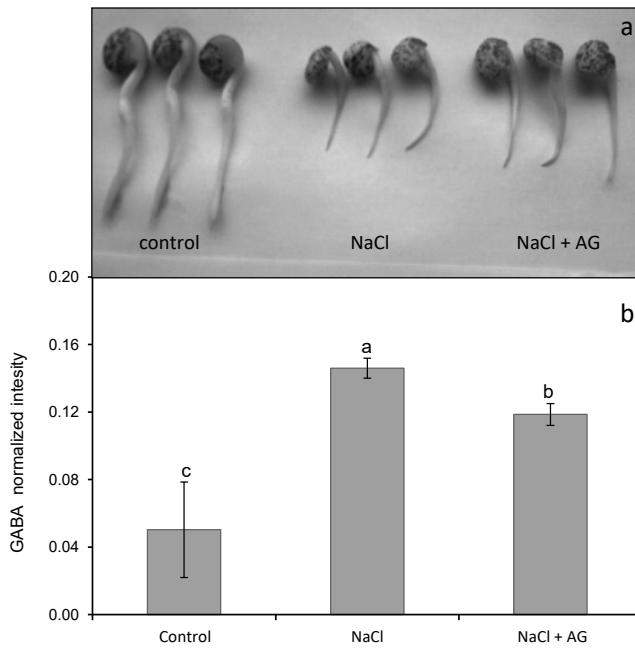


Fig. 4 The content of GABA in the roots of 3-day-old lupine seedlings incubated in HS (control) and HS with 200 mM NaCl in the absence or presence of 1 mM AG for 24 h in the dark. Data were transferred to Excel and mean values with standard deviation were calculated for GABA content in the samples. In the upper part of the figure, there is a photograph of the seedlings after incubation in conditions described above.

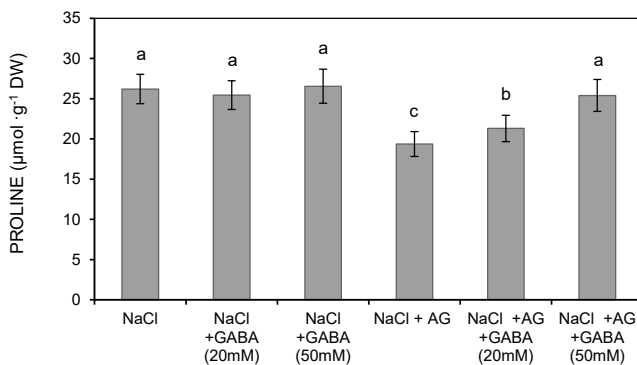


Fig. 5 The content of proline in the roots of 3-day-old lupine seedlings incubated in HS (control) and HS with 200 mM NaCl in the absence or presence of 1 mM AG, 20 or 50 mM GABA for 24 h in the dark. The values are the means of three replicates of measurements obtained in four independent experiments. The differences in the measured parameters were analyzed for statistical significance using one-way analysis of variance (ANOVA) and the Tukey–Kramer multiple comparison test ($n = 9$, $p < 0.01$). The same letter on the bars indicates that there were no significant differences between the means.

seedlings incubated in NaCl together with AG restored Pro content to the level observed in NaCl-treated seedlings (Fig. 5).

Discussion

In this research, we demonstrate for the first time a close relationship between Put catabolism in relation to Pro and GABA accumulation in the roots of 2-day-old lupine seedlings growing under salt stress for 24 h. In the roots of lupine seedlings treated with NaCl, Pro content increased significantly in comparison to the control and, at the same time, the reduction in Put and Spd levels was observed. In our model, in the lupine roots, we were able to see this negative correlation because Pro transport to a shoot was very limited as this part of a plant had not yet been developed.

It is interesting to note that the changes in cellular PAs and Pro level often seem to occur in a coordinated manner even through their biosynthesis shares a common precursor, i.e., Glu [9,35,36]. Additionally, Glu is used in rapid GABA accumulation in cytoplasm in response to many stresses [5,16,19]. However, it is not always clear how Pro synthesis proceeds – directly from Glu by Δ -pyrroline-5-carboxylate synthetase (P5CS) or from ornithine (Orn) by Orn aminotransferase (OAT). It was shown that Pro is made preferentially via Orn under normal conditions, whereas the Glu pathways account for major Pro accumulation during osmotic stress [35,37]. Therefore, the high Pro level detected in lupine root of 3-day-old seedlings growing under salt stress could be a consequence of channeling Glu and/or Orn preferentially to this route, resulting in a complete control of substrate availability for Pro biosynthesis. Another reason for a decrease in Put content in plants growing under salt stress might be an increase in DAO activity [9,20,22] and/or decrease in arginine decarboxylase activity [15]. DAO catalyzes oxidation of Put and cadaverine, and with lower affinity also Spd and Spm [38]. In the roots of lupine seedlings growing under salt stress, we observed a significant increase in DAO activity. We assume then that the decrease in Put content observed in the roots of lupine growing under salt stress was a result of DAO action. This was additionally confirmed by using AG, a specific DAO inhibitor, which completely restored the Put content to the control level.

Interestingly, in the presence of AG the Pro content in the roots of lupine seedlings growing under salt stress was lower than in roots of salt-treated seedlings. This result suggests that catabolism of Put in lupine roots

is indirectly involved in Pro accumulation under NaCl stress. Similarly, in the soybean leaves growing in salinity conditions quantitative contribution of Put degradation to Pro formation was between 15 and 20% [22]. In other papers, the antithetic trends of Put and Pro, namely an increase in Pro associated with a decrease in Put or other PAs, were described as a short-term response to high salt concentrations and this physiological finding was positively related to salt tolerance [9,11,20,22]. No drastic and continuous accumulation of Pro parallel to PAs decrease after prolonged salt stress was observed

[20]. The foregoing studies were conducted on leaves and/or callus cells, and in literature there is no similar report regarding roots.

PAs are assumed to perform their function through their oxidative products such as GABA. Put and Spd catabolism generates H_2O_2 and GABA. An increase in Put degradation via DAO activity under salt stress contributes both to GABA and Pro accumulation [21,22].

GABA, like Pro, is also synthesized from Glu by Glu decarboxylase [16,39]. Increased flux of Glu through this set of reactions certainly causes a major loss of cellular Glu, which is not tolerable if cells must continue other metabolic functions involving Glu: biosynthesis of proteins and other amino acids. Osmotic stress and other stresses cause a strong conversion of Glu into both GABA and Pro in cytoplasm and from that, these compounds compete for the same substrate, Glu. As mentioned before, GABA is also produced from Put and Spd oxidation by DAO and PAO. 4-Aminobutanal produced in Put catabolism by DAO and terminal catabolism of Spd can be converted to GABA [21,40]. There is only limited information on GABA accumulation derived from polyamine degradation under salt stress. It was reported that PA degradation pathway supplied about 39% GABA in soybean seedlings roots under NaCl stress [21]. Similarly, one-third of GABA formation in germinating fava bean under salt stress derived from PA degradation pathway [23].

In our investigation it was shown that in the roots of lupine seedlings growing under salt stress in the presence of AG, the level of GABA decreased by about 22%. It means that Put degradation takes part in GABA accumulation. Additionally, we observed that the roots of seedlings incubated in NaCl were significantly shorter than the control, and if AG was added to the seedlings incubated in NaCl, the roots of plants were longer than in NaCl alone. It means that higher level of GABA inhibits roots elongation. This thesis is supported by Renault et al. [41] findings, who observed growth inhibition of primary roots and dark-grown hypocotyl in *A. thaliana* as the result of decreased expression of the genes encoding secreted and cell wall-related proteins. Also Batushansky et al. [42] showed that expression of genes associated with cell wall was inhibited in seedlings of *Arabidopsis thaliana* by exogenous GABA.

The involvement of DAO-mediated Put oxidation process in GABA production has been recently suggested by Sobieszczuk-Nowicka et al. [43]. Microarray-based profiling of glutamate decarboxylase gene expression has shown that in senescing barley leaves GABA, synthesis from glutamate is gradually suppressed. Put oxidation then becomes an alternative source of GABA to the tricarboxylic acid cycle and also for some as yet undefined signaling pathways [43]. This GABA pool could be involved indirectly in the synthesis of Glu and further synthesis of Pro from Glu [16,39].

GABA production deriving from PA degradation enriches the GABA pool in cytoplasm, which is mainly synthesized there from Glu, therefore more Glu can be

used to Pro synthesis. This thesis was confirmed by experiments in which adding AG to the lupine seedlings growing for 24 h under salt stress resulted in a decrease of GABA level and, indirectly, Pro content in roots, as compared to NaCl treatment alone. The exogenous GABA added to this variant restored Pro content to its level observed in NaCl only. Both these interdependences can partially explain the regulatory link between polyamine catabolism and proline accumulation shown in Fig. 6.

Contrary to the lower level of Put and Spd, a significant increase in Spm was observed under salinity condition. This seems to represent a general feature of plant responses to salinity [11,15]. Spm can be involved in stabilization of nucleic acid structure [44] and can operate as a free radical scavenger [45,46]. It was observed that Spm effectively prevented lipid peroxidation in senescing oats leaves [47], in sunflower leaf discs treated with paraquat [48], or in bean leaves treated with

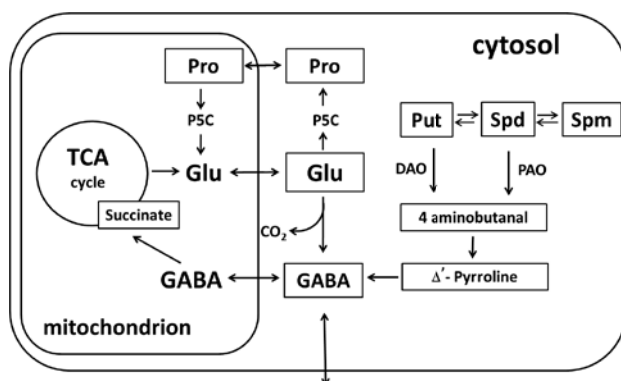


Fig. 6 Relationship between PA catabolism and Pro and GABA accumulation in the roots under salt stress. Pro and GABA compete for the same substrate – Glu. GABA produced from Put and Spd degradation increased its pool in cytosol, therefore more Glu can be converted to Pro. Abbreviations: PA – polyamine; Pro – proline; P5C – 1-pyrroline-5-carboxylate; DAO – diamine oxidase; PAO – polyamine oxidase; TCA cycle – tricarboxylic acid cycle.

acid-rain [49]. Conclusive evidence regarding antioxidant roles of PAs was provided by Nayyar and Chandler [50] and Legocka et al. [45], who observed that exogenously applied Spm reduced H₂O₂ and MDA contents in chickpea plants exposed to drought and cold stress and in greening barley leaves exposed to lead stress.

Conclusions

The present study provides a basis for a better understanding of the role played by PAs in plant response to salt stress, and establishes PA metabolism as an important regulatory mechanism in Pro accumulation. The roots of 2-day-old seedlings of lupine were used in experiments. In these plants, shoots were not yet developed, and an export of Pro from roots to shoots was for that reason minimal. Application of AG to the seedlings growing under salt stress showed a direct link between Put catabolism and Pro and GABA accumulation in roots. An inhibitor of DAO activity, AG, added to NaCl-treated seedlings lowered Pro and GABA content in roots exposed to NaCl, and addition of exogenous GABA to this variant restored Pro level. We report for the first time a correlation between a decline in Put content and an increase in Pro and GABA levels in roots of lupine seedlings growing under high salinity. The regulatory mechanism of Pro accumulation in part as a result of Put degradation indicates an indirect link to other compound of PA catabolism, such as GABA. The GABA derived from PA degradation enriches pool of GABA in cytosol, which is intensively synthesized during stress from Glu. Therefore, more Glu can be converted to Pro, the level of which also rapidly increases under high salinity.

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