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Effects of Auxin-Type Plant Growth Regulators and Cold Stress on the Endogenous Polyamines in Pea Plants

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Abstract: The effect of pre-application of structural auxin analogues TA-12 (1-[2-chloroethoxycarbonyl-methyl]-4-naphthalenesulfonic acid calcium salt) and TA-14 (1-[2-dimethylaminoethoxicarbonylmethyl] naphthalenechlormethylate) on biochemical parameters of pea (*Pisum sativum* L. cv. Jablo) plants subjected to low temperature (LT, −1 °C, for 24h) was studied. For the first time the effects of these auxin analogues, applied with or without LT were investigated on the endogenous polyamine (PA) content. The LT treatment increased free and bound putrescine (Put) and spermine (Spm), conjugated and bound spermidine (Spd), accompanied by a decrease in conjugated Put and Spm, and free Spd. Stress biomarkers hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) as well as proline were augmented by LT treatment. The TAs application decreased conjugated polyamines (Put, Spm and Spd), free Spd, H_2O_2 and MDA but increased bound Spm and proline in pea plants. The application of TAs before LT lessened the alterations in PAs (mainly in free and bound fractions) and stress biomarkers content caused by LT, and enhanced conjugated Spd and phenolics, which contributed to increased plant cold tolerance.

Keywords: low temperature treatment; *Pisum sativum* L.; auxin analogues; polyamines; stress markers

Citation: Jankovska-Bortkevič, E.; Katerova, Z.; Todorova, D.; Jankauskienė, J.; Mockevičiūtė, R.; Sergiev, I.; Jurkonienė, S. Effects of Auxin-Type Plant Growth Regulators and Cold Stress on the Endogenous Polyamines in Pea Plants. *Horticulturae* **2023**, *9*, 244. https://doi.org/10.3390/horticulturae9020244

Academic Editors: Maria G. Maleva, Alexander A. Ermoshin and Galina Borisova

Received: 28 December 2022
Revised: 8 February 2023
Accepted: 9 February 2023
Published: 10 February 2023

1. Introduction

Pea (*Pisum sativum* L.) is grown for human and animal consumption. This crop is recognised as an inexpensive valuable source of protein, carbohydrates, fibres, minerals and vitamins [1]. Most pea varieties are cultivated during spring season when the average temperature is around 20 °C [2]. *P. sativum*, cv. Javlo is a pea variety that has short to medium stem length and medium plant height. The plant cultivar is early ripening; its average duration of vegetation is 77 days (55–65 days pass on average from germination to harvest).

Low temperature or cold stress includes chilling (0–15 °C) stress and freezing (below 0 °C) stress [3]. A sudden drop in air temperatures at or just below 0 °C causes frost. Frost occurs during spring in temperate regions and frost events could happen frequently [4,5]. Productivity of pea depends on plants' ability to acclimate, and sudden frost is the main reason for reduction in yield [6,7]. Similarly to other extreme climate changes, cold usually induces damage in cells, macromolecules (proteins, nucleic acids) and promotes osmotic and oxidative stress [5,8]. Consequently, it initiates stress signalling — activation of various mechanisms of stress related signals (phytohormonal, secondary metabolite biosynthesis, etc.), aiming to restore homeostasis (by protection and repair of proteins, nucleic acids, membranes). The low-temperature tolerance in plants is possible if the mentioned steps have adequate response, i.e., the overproduction of reactive oxygen species (ROS: hydrogen peroxide, superoxide, hydroxyl radical, singlet oxygen etc.), do not occur, and plants

successfully restore their homeostasis. In addition to ROS, calcium (Ca^{2+}) signalling, depending on the cell concentration could also operate as a secondary messenger to fine-tune different physiological processes [3]. Moreover, the interaction between Ca^{2+} signalling, ROS and phytohormones is known to be involved during cold stress [9]. Phytohormonal changes and their crosstalk are known to occur during cold acclimation and auxins and polyamines (PAs) are implicated as well [3,10,11]. Putrescine (Put), spermidine (Spd) and spermine (Spm) are the most widespread polyamines among plants, containing two, three or four amino groups. Their positive charge due to amino groups, allow them to bind to the negatively charged groups of membrane phospholipids or nucleic acids shaping plants' response to stress, including low-temperature [12]. These plant growth regulators appear as free molecules, conjugated with phenolic acids (or other small molecules), or bound to macromolecules [13]. Under stress conditions, polyamines act as scavenger of oxygen radicals, serve as compatible solutes, stabilise macromolecules and cellular biomembranes and participate in other stress-related plant responses [14].

The modulation of plant's antioxidative system usually has positive effect on its tolerance to stress [15]. One of the strategies to improve antioxidant defence system is the so called 'priming', which can be achieved using diverse tools—application of mild stressor, chemical or biological agents. Chemical priming is known as positive physiological defence response caused by various compounds which stimulate adaptive cellular response to a stressor [16–18]. Priming stimulates acquisition of resistance to the applied stressor by initiation of multiple signalling pathways (as hormonal; induction of ROS as signalling molecules; etc.), induction of biosynthesis of secondary metabolites, activation of antioxidant defence system, etc.

Auxins influence multiple aspects of plant growth and development, including plant response to environmental stresses as low temperature. The structural analogues of naphthyl acetic acid i.e., 1-[2-chloroethoxycarbonyl-methyl]-4-naphthalenesulfonic acid calcium salt (TA-12) and 1-[2-dimethylaminoethoxicarbonylmethyl] naphthalenechlormethylate (TA-14) were used in the current study. These auxin analogues were analysed earlier as promising protectors against diverse stress factors — cold stress in *Brassica napus* [19–21]; high temperature [22] and herbicidal stress [23,24] in *P. sativum* plants; PEG-induced drought in pea [25], maize and wheat [26]. The focus of the current study is on the modulation capacity of TAs pre-application on pea physiological responses to low-temperature stress. The effect was monitored through the alterations of PAs (free, conjugated and bound Spm, Spd and Put), which is the first communication regarding the influence of exogenous auxin analogues on endogenous polyamines in pea plants. In addition, well-known oxidative stress biomarkers (malondialdehyde (MDA), hydrogen peroxide), phenolics, proline and photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) were also studied.

2. Materials and Methods

2.1. Plant Material and Treatments

P. sativum plants (cv. Javlo) were grown in phytotron chamber at the Laboratory of Plant Physiology, Nature Research Centre, Vilnius, Lithuania on substrate composed of garden compost and peat moss (1:1 *v/v*) under controlled conditions: 21 ± 1 °C temperature, 16/8 h photoperiod, 150 µmol m^{-2} s^{-1} photon flux density. The seeds were cultivated in plastic cube pots of 40 cm edge length, each pot contained 10 seeds. Plants were cultivated for 21 days until BBCH 14–15 stage (corresponding to the phenological growth stage: 4 to 5 unfolded leaves with stipules or 4 to 5 developed tendrils). Twenty one day-old seedlings were sprayed with water solutions of 1 mM TA-12 (1-[2-chloroethoxycarbonyl-methyl]-4-naphthalenesulfonic acid calcium salt) and TA-14 (1-[2-dimethylaminoethoxycarbonylmethyl]naphthalene chlormethylate). The control plants were sprayed with distilled water. After 24 h, half part of the pots with seedlings (control and treated plants with auxin analogues TA-12 and TA-14) were subjected to low temperature (−1 °C, LT) stress for 24 h; other group of pots with seedling continued to grow under

control conditions. Each treatment group consisted of three pots. For the biochemical analyses, plant material of stipules of 22-day-old peas was collected.

2.2. Content of Oxidative Stress Biomarkers MDA and Hydrogen Peroxide (H_2O_2)

For the analysis of the amount of MDA and hydrogen peroxide, fresh plant material was homogenised with 5 % (*w/v*) trichloroacetic acid (TCA). The homogenates were centrifuged 12,000× *g* 20 min (refrigerating centrifuge MPW-351 R, MPW Med. instruments, Warsaw, Poland).

MDA was determined as follows: the supernatant (2 mL) was mixed with 3 mL TCA containing 0.5 % 2-thiobarbituric acid. Samples were incubated 30 min at 95 °C, and subsequently cooled on ice. MDA concentrations were determined at 600 and 532 nm, using absorbance coefficient of 155 mM^{-1} cm^{-1} [27].

Hydrogen peroxide was determined according to the method of Alexieva et al. [28] with slight modifications: 0.5 mL supernatant was supplemented with 0.5 mL potassium phosphate buffer (10 mM, pH 7.0) and 1.0 mL 1M potassium iodide. The mixture was incubated at 25 °C for 30 min in the dark. The absorbance was measured at 390 nm wavelength and calculated using standard curve based on known concentrations of (H_2O_2).

The spectrophotometrical measurements were made using SPECORD 210 Plus (Analytik Jena GmbH, Jena, Germany).

2.3. Determination of Proline Content

Plant material (0.5 g) was homogenised for 2 min with 10 mL of 3% sulfosalicylic acid solution. After homogenisation, the supernatant was extracted for 14 h at 4 °C, and the obtained extracts were centrifuged at 700× *g* for 20 min (centrifuge MPW–351 R, MPW Med. instruments, Warsaw, Poland). Acidified ninhydrin solution was prepared by dissolving ninhydrin (1.25 g) (Roth, Karlsruhe, Germany) in glacial acetic acid (30 mL) (Roth, Karlsruhe, Germany) and 6 M phosphoric acid (20 mL). The supernatant (2 mL) was mixed with equal parts of acetic acid and acidified ninhydrin reagent [29]. The obtained mixtures were heated in an oven (Kleinfeld Labortechnik, Gehrden, Germany) for 60 min at 105 °C, placed to cool down in an ice bath for 15 min. Then 2 mL of toluene was added and 1 h later the absorbance was read at 520 nm by spectrophotometer using a quartz multicuvette (Hellma, Jena, Germany) and Rainbow microplate reader (SLT Labinstruments, Rendsburg-Eckernförde, Germany). Toluene was used as a blank. Results were expressed as μmol of proline per g of fresh weight (FW).

2.4. Determination of Total Phenolics Content

Plant material (0.3 g) was homogenised with 2 mL of 5 % TCA. After centrifugation at 3000× *g* (4 °C) for 15 min, the resultant supernatants were incubated with Folin-Ciocalteu reagent for 2 h at room temperature [30]. The absorbance was read at 725 nm on a Multiskan Spectrum spectrophotometer with microplate reader (Thermo Electron Corporation, Vantaa, Finland). Gallic acid was used as a standard to calculate the results.

2.5. Analyses of Polyamine Content

Three fractions of the major polyamines putrescine, spermidine and spermine were determined after extraction of 0.3 g plant material with 5% TCA and centrifugation at 3000× *g* (4 °C) for 15 min. Free polyamines were analysed in the supernatant according to the method of Smith and Best [31] by direct dansylation. The conjugated PAs were measured in HCl-hydrolysed supernatant (soluble bound polyamines) and bound PAs in HCl-hydrolysed pellets (insoluble bound polyamines) according to the method of Torrigiani et al. [32]. After hydrolysis, both conjugated and bound PAs were dansylated and processed following the procedure for free polyamines. The Put, Spd and Spm were separated by thin layer chromatography using precoated plates of Silicagel G 60 (Merck KGaA, Darmstadt, Germany) and developed in system of solvents cyclohexane:ethylacetate (3:2 *v/v*). Spots were visualised under UV light, then scraped off from plates and eluted

in 2 mL anhydrous acetone. The fluorescence was measured on Spectrofluorophotometer RF-1601 (Shimadzu, Kyoto, Japan), at excitation wavelength 360 nm and at emission wavelength 505.5 nm. The fluorescence data of plant samples were compared with dansylated polyamine standards on the same plate and expressed as nmol per g FW of plant material.

2.6. Analyses of Chlorophyll and Carotenoids Contents

The photosynthetic pigments were extracted from 0.05 g of fresh material in 3 mL of N,N′-dimethylformamide. The absorption was measured at 480, 664, 647 nm wavelengths on SPECORD 210 Plus (Analytik Jena GmbH, Jena, Germany). Chlorophylls *a* and *b*, and carotenoids concentrations were calculated according to Wellburn [33].

2.7. Statistical Analysis

The results presented are obtained from three independent biological experiments. The samples were collected in two replicates each. The data are mean values ± standard error (SE). To evaluate the significant differences between treatments, one-way ANOVA with post-hoc Duncan's multiple range test was used ($p < 0.05$).

3. Results

3.1. Content of Oxidative Stress Markers Malondialdehyde and Hydrogen Peroxide

The contents of MDA and hydrogen peroxide in pea plants are presented in Figure 1. Slight decrease (by 20%) in MDA was observed due to TA-12 and TA-14 applications (Figure 1A). In contrary, an increase by 30% was found in pea after LT treatment. After combined application of Tas + LT, MDA content decreased significantly as compared to LT treatment. The values of MDA due to combined application became closer to the control value than those, obtained after LT alone treatment, but only TA-14 + LT reached the control levels.

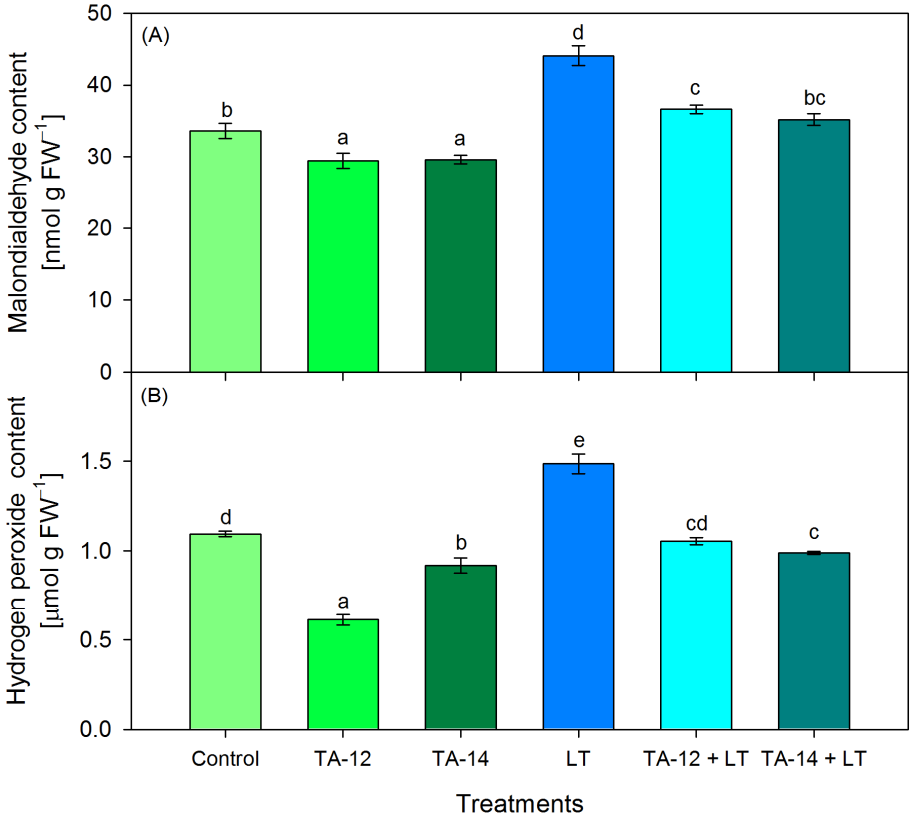

Figure 1. Content of malondialdehyde (MDA) (**A**) and hydrogen peroxide (**B**) in pea sprayed with TA-12 or TA-14 and subjected to low temperature (−1 °C, LT) for 24 h. Data are mean values ± SE. Different letters designate statistically significant difference at $p < 0.05$.

A decline in H_2O_2 content (Figure 1B) was found after TA-12 (by 40%) and TA-14 (by 20%) application. An opposite trend (raise by 40%) was observed due to LT treatment. The combined application reduced H_2O_2 content as compared to LT treatment and neared the control values.

3.2. Content of Proline and Total Phenolics

Proline content (Figure 2A) was increased considerably due to TA-12 and TA-14, by 50% and 80%, respectively. It was increased even more (by 140%) after LT treatment. After combined application it was reduced significantly due to TA-12 + LT (by 30%) as compared to LT treatment but not significantly due to TA-14 + LT (by 17%). Total phenolics content (Figure 2B) was not changed significantly after individual treatments with TAs or LT. An increment in the phenolics content was observed due to TA-12 + LT (by 20%) and TA-14 + LT (by 40%).

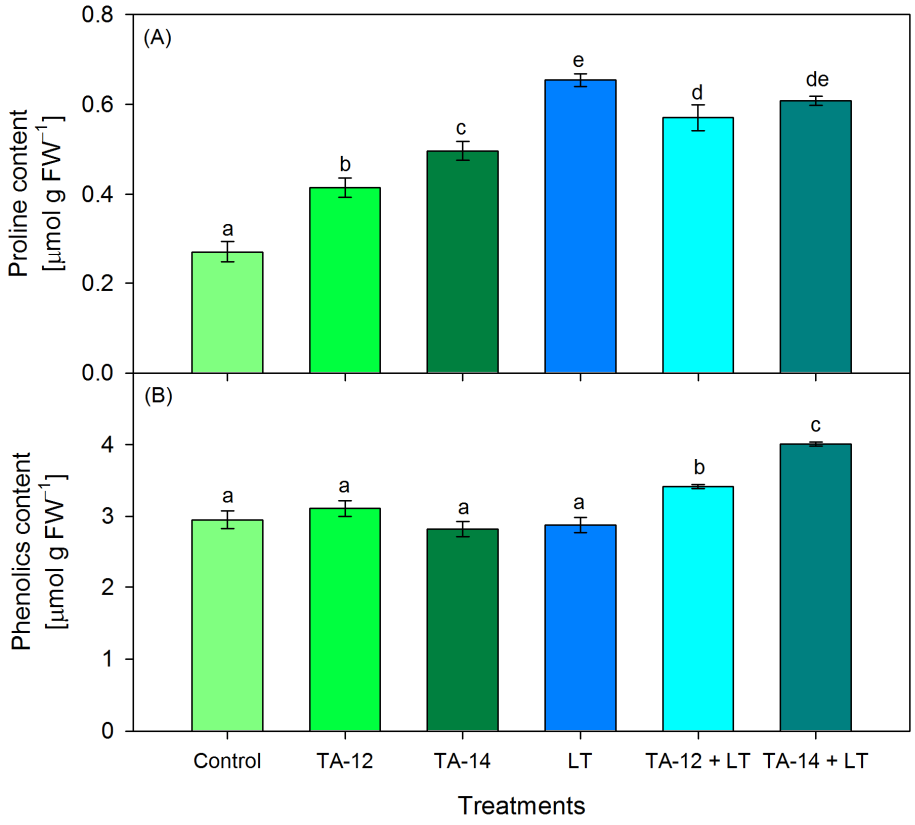

Figure 2. Content of proline (**A**) and total phenolics (**B**) in pea sprayed with TA-12 or TA-14 and subjected to low temperature (−1 °C, LT) for 24 h. Data are mean values ± SE. Different letters designate statistically significant difference at $p < 0.05$.

3.3. Content of Free, Conjugated and Bound PAs (Putrescine, Spermidine and Spermine)

The content of free, conjugated and bound Put is presented in Figure 3. An increase (by 30%) in free Put content (Figure 3A) was observed due to LT treatment but combined application of TAs + LT restored the control values. The content of conjugated Put (Figure 3B) was substantially decreased after all treatments, namely TA-12 (by 80%), TA-14 (by 90%), LT (by 40%), TA-12 + LT (by 60%) and TA-14 + LT (by 90%). The content of bound Put (Figure 3C) was increased substantially only due to LT treatment (by 40%). It was reduced significantly due to combined applications as compared to LT treatment but reached the control values only after TA-14 + LT.

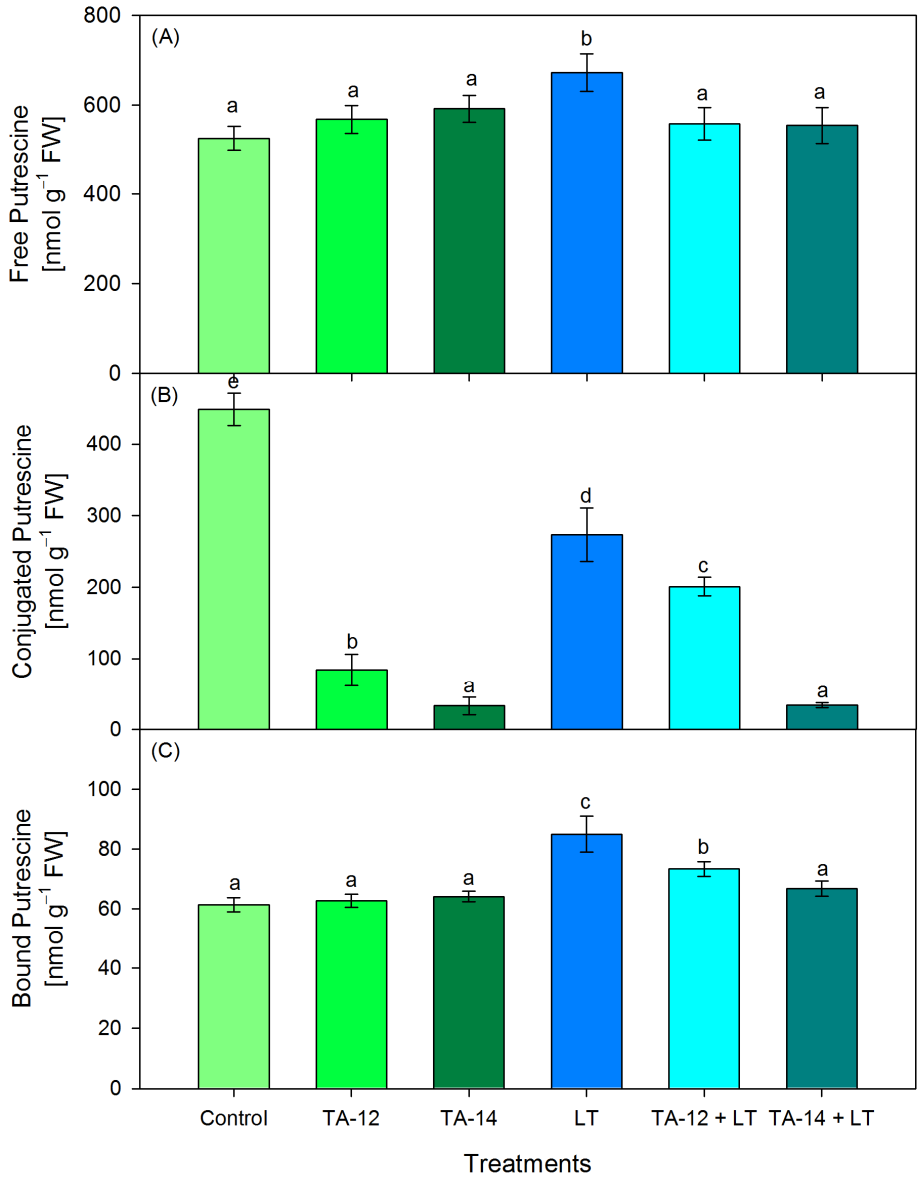

Figure 3. Content of free (**A**), conjugated (**B**) and bound (**C**) putrescine (Put) in pea sprayed with TA-12 or TA-14 and subjected to low temperature (−1 °C, LT) for 24 h. Data are mean values ± SE. Different letters designate statistically significant difference at $p < 0.05$.

The content of free, conjugated and bound Spd is presented in Figure 4. A slight decrease in free Spd (Figure 4A) was found after TAs and TAs + LT applications. An opposite tendency was observed after LT treatment. The conjugated Spd content (Figure 4B) declined due to the individual application of TAs (by 50 and by 70% after TA-12 and TA-14, respectively) but increased considerably by 160% after LT treatment. The combined application of TAs + LT reduced significantly by 70% its content as compared to LT treatment, but remained two-fold higher than the control values. The bound Spd content (Figure 4C) was significantly incremented by 50% only after LT treatment and combined TAs + LT application restored control values.

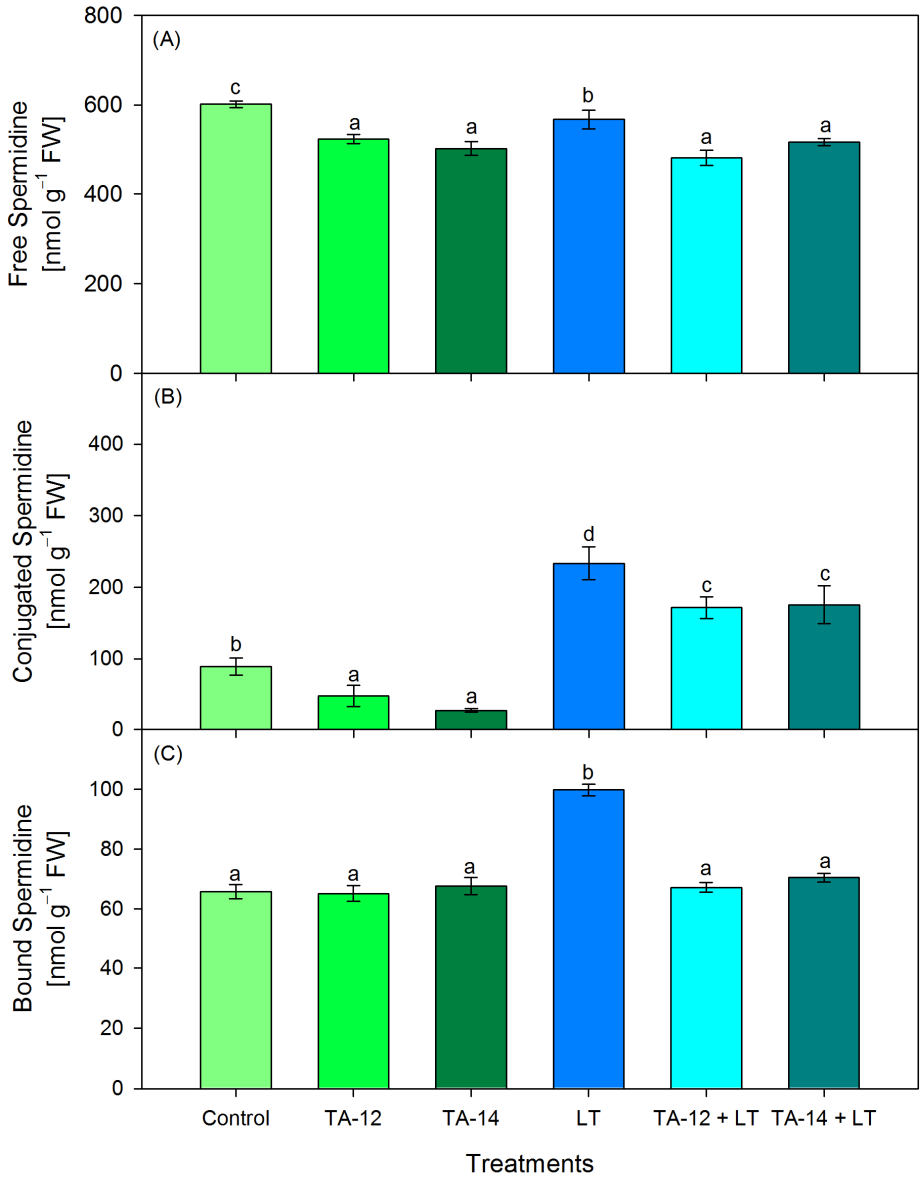

Figure 4. Content of free (**A**), conjugated (**B**) and bound (**C**) spermidine (Spd) in pea sprayed with TA-12 or TA-14 and subjected to low temperature (−1 °C, LT) for 24 h. Data are mean values ± SE. Different letters designate statistically significant difference at $p < 0.05$.

The content of free, conjugated and bound Spm is shown in Figure 5. An increased content of free Spm (Figure 5A) was found after LT treatment but combined application of TAs + LT restored control values. All applied treatments caused substantial decrement in the content of conjugated Spm (Figure 5B) reaching minimal value after application of TA-14 alone (by 48%) or in combination with LT (by 63%). The content of bound Spm (Figure 5C) was increased after alone TA-12 (by 23%) and TA-14 (by 20%) applications, as well as after LT (by 26%) treatment. Combined application of TAs + LT restored bound Spm to the control values.

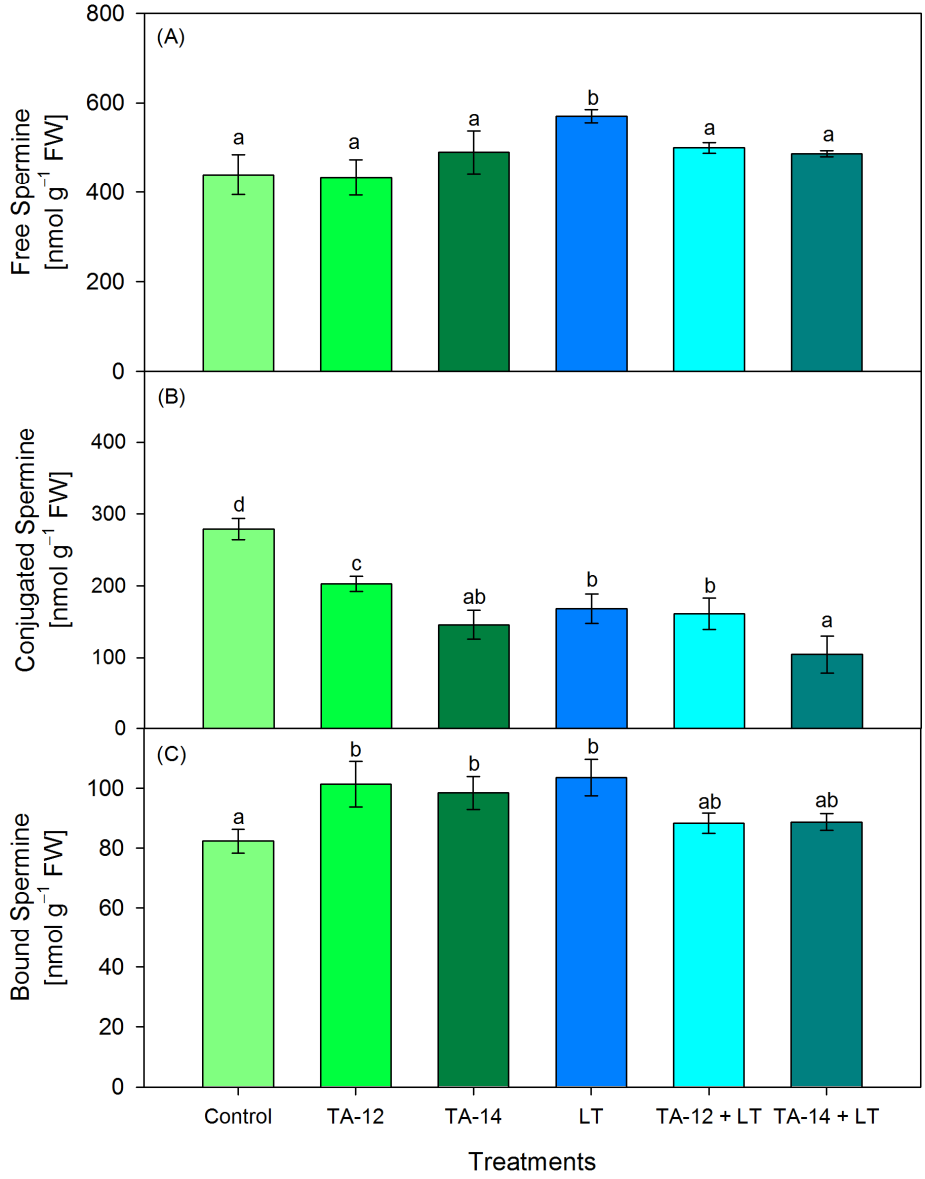

Figure 5. Content of free (**A**), conjugated (**B**) and bound (**C**) spermine (Spm) in pea sprayed with TA-12 or TA-14 and subjected to low temperature (−1 °C, LT) for 24 h. Data are mean values ± SE. Different letters designate statistically significant difference at $p < 0.05$.

Total putrescine, spermidine and spermine content calculated as the sum of free, conjugated and bound fractions are presented in Table 1. We found that LT treatment caused an increase in individual PAs content, while TAs compounds reduced total PAs when applied alone or before LT stress. Total Spd after TAs + LT treatment was alike to control level.

3.4. Content of Photosynthetic Pigments

The content of chlorophylls and carotenoids is shown in Figure 6. The low temperature did not alter contents of the photosynthetic pigments. The content of chlorophyll *a* was augmented by 50% due to TA-14 (Figure 6A). It was also increased considerably by 50% and 70% after combined application of TA-12 + LT and TA-14 + LT, respectively. Similar tendency was observed for chlorophyll *b* content: an increase by 40%, by 40%, and by 60% due to TA-14, TA-12 + LT and TA-14 + LT application, respectively (Figure 6B). The content of carotenoids (Figure 6C) was not altered significantly, except due to TA-14 + LT (raise by 50%).

Table 1. Total content of putrescine (Put), spermidine (Spd) and spermine (Spm) in pea sprayed with TA-12 or TA-14 and subjected to low temperature (−1 °C, LT) for 24 h.

Treatments	Total Put, nmol g^{-1} FW	Total Spd, nmol g^{-1} FW	Total Spm, nmol g^{-1} FW
Control	1037 (100 *)	756 (100)	800 (100)
TA-12	750 (72)	636 (84)	738 (92)
TA-14	689 (66)	597 (79)	734 (92)
LT	1230 (119)	930 (123)	842 (105)
TA-12 + LT	853 (82)	721 (95)	750 (94)
TA-14 + LT	655 (63)	763 (101)	680 (85)

* Digital in brackets stands for percent to the control.

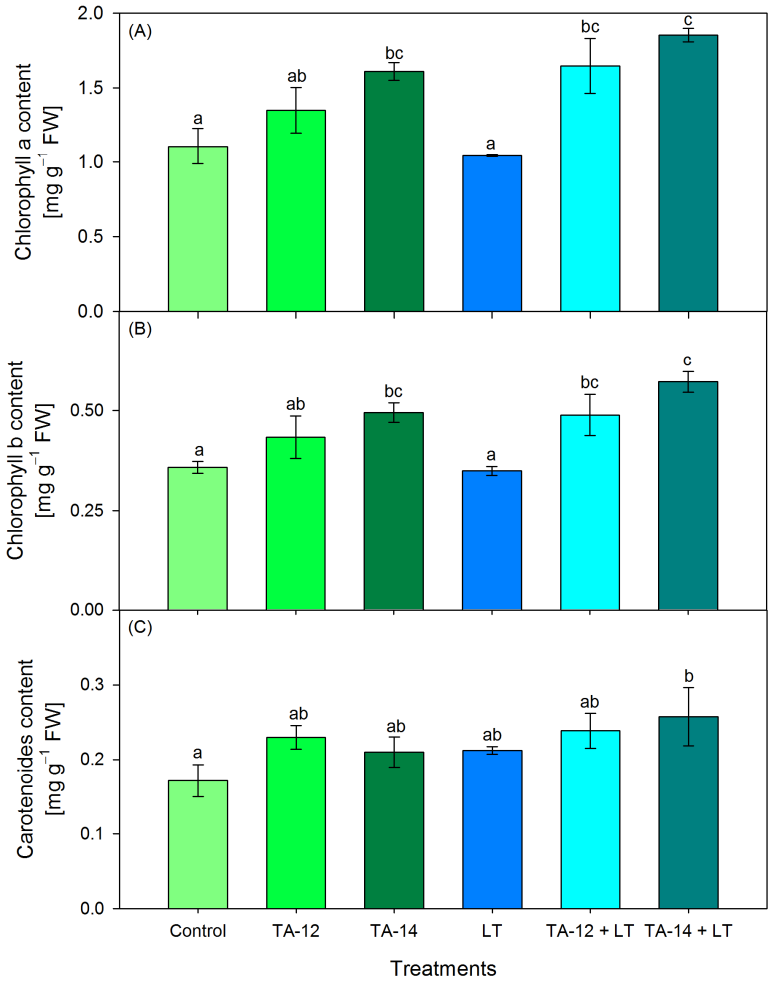

Figure 6. Content of chlorophyll *a* (**A**), chlorophyll *b* (**B**) and carotenoids (**C**) in pea sprayed with TA-12 or TA-14 and subjected to low temperature (−1 °C, LT) for 24 h. Data are mean values ± SE. Different letters designate statistically significant difference at $p < 0.05$.

4. Discussion

Cold induces a wide range of physiological, biochemical, genetic and epigenetic alterations. Plant responses to LT stress is attributed to changes in photosynthesis, phytohormonal balance, biomembranes' fluidity and integrity, ROS signalling, antioxidant defence, production of specific stress proteins, compatible solutes, etc., [3,18,34]. Among the parameters studied one of the first alterations might be ROS formation and membrane damage, followed by osmolytes (proline) accumulation and ROS control (PAs, phenolics), and finally the photosynthetic pigments. The increased MDA content due to LT demonstrated that membrane damages and oxidative stress are taking place, as it was found along

with the increase of the content of H_2O_2. Our data are in line with the results obtained in other studies [35–39] where cold stress caused an accumulation of stress biomarkers, signifying for presence of oxidative stress events and membrane deteriorations. To withstand LT stress, a series of sophisticated physiological and biochemical events are involved, such as synthesis of substances that participate in the regulation of cell osmotic potential, stabilisation of cell biomembranes and scavenging of ROS in cold stressed plants [34]. Proline is known not only as an abiotic stress biomarker, but also as a ROS scavenger and osmoprotectant [40]. The accumulation of proline, along with that of other cellular compatible solutes as amino acids, betaines and sugars, plays a key role in the stress-counteracting mechanisms of plants subjected to low-temperature conditions [41]. Expectedly, we found a substantial increase of proline content due to LT stress, which is in line with the findings of other research [36,39].

Although the total phenolics are considered as effective antioxidants and ROS scavengers against diverse abiotic stress conditions [42] and usually are increased due to stress, Król et al. [43] reported a reduction in their concentration in grapevine leaves subjected to cold. We found no changes in phenolics level due to LT treatment. In addition, the cold stress applied did not lead to significant changes in photosynthetic pigments, and particularly in carotenoids, probably as the intensity (-1 °C) and duration (24 h) of the applied stress were not so strong to pea, which relatively tolerates low temperatures.

Polyamine content was also altered due to LT stress. Total polyamines increased, which was a consequence of an increase of free and bound fractions. The accompanied decrease in conjugated Put and Spm suggests a release of the conjugated into free form. Considerable changes were observed in conjugated Spd also, which was significantly increased due to LT — more than it was anticipated as conversion from free fraction. While the increased Put is regarded as stress inducible biomarker rather than cellular protectant, increased Spd and Spm levels due to stress are considered to be associated with better plant stress tolerance [44]. Because of the superior number of positively charged amino groups, Spm and Spd are more efficient than Put in stabilisation of the cell biomembranes and cell organelles via linkage with their negatively charged functional groups [13]. Overexpression of Spd synthase in Arabidopsis resulted in a considerable increase in Spd content in leaves and enhanced tolerance to chilling and even freezing temperatures [45]. Shen et al. [46] proposed also that Spd takes part in the chilling tolerance of cucumber. In addition, Spm can preserve DNA molecules under stress conditions, and can serve as a direct free radical scavenger [47].

The role of auxins in plant's physiological reactions to cold stress was not extensively studied; however, different authors showed the importance of high endogenous auxin levels for cold tolerance in plants [21,48,49]. Garbero et al. [48] reported that the cold tolerance of *Digitaria eriantha* is associated with a rise of IAA amount and with stability of cytokinin levels. However, short-term cold caused a reduction of IAA metabolites levels in arabidopsis [49]. Jankauskiene et al. [21] reported that at the end of cold acclimation period, the IAA conjugates were augmented while free IAA levels were reduced in oilseed rape cultivars. In addition, the authors showed that TA-12 and TA-14 applications increased the formation of IAA conjugates at the end of cold acclimation period. Here, for the first time we studied the effect of auxin-type PGR (TA-12 and TA-14), applied alone or in combination with low temperature on pea plants. Until now, the effect of exogenous auxin analogues on endogenous level of polyamines in pea plants was not studied. In the current study we illuminate the role of diverse polyamines fractions in auxin's and low temperature stress responses in pea plants.

We found that single TAs application did not change noticeably free and bound Put, Spd and Spm, while conjugated fraction was strongly reduced, which reflected on decreased total amount of PAs. Meanwhile proline content in TAs-treated plants increased considerably. Polyamines and proline share common precursor (glutamate) in their biosynthesis [50]. Therefore, the general drop of PAs observed in TAs-treated pea plants correlates well with the increased level of proline. Similar tendencies of PAs alterations in free and

bound fractions were observed when TAs were applied prior to LT stress. However, conjugated Spd was increased significantly in TAs + LT plants, which reflected on maintenance of total Spd near the control level. It should be noted that at the same time total phenolics content did not change after single TAs application while it was increased due to TAs + LT treatment. Since the total phenolics pool contain hydroxycinnamic acids, which are main derivatives that conjugate with PAs [13], it could be speculated that the content of conjugated PAs increased in parallel with the increment of phenolics compounds due to TAs + LT application. This probably reflects on enhanced cold tolerance in TAs + LT-treated plants.

Interestingly, only the application of TA-14 caused a raise in pea chlorophylls. Such chlorophyll stimulation was reported earlier due to exogenous application of different IAA concentrations in *Brassica juncea* [51]. Similarly, Cl-substituted auxin derivatives were reported to increase chlorophyll concentrations [52]. The combined application of TAs + LT raised chlorophyll *a* and chlorophyll *b* concentrations higher than the control, which might be explained by the auxin nature of TAs.

One of the structural differences between TA-12 and TA-14 is that TA-12 is a calcium salt. Therefore, the role of Ca^{2+} signalling as second messenger may clarify the slight differences in pea plant response to TAs application. For example, the H_2O_2 decrement due to TA-12 was found to be more intensive than due to TA-14, which might be explained by the fact that H_2O_2 also plays a role as signalling molecule [53]. In addition, it is well documented that exogenous auxin is able to decrease H_2O_2 level via induction of gene expression and enhancement of the activity of H_2O_2 scavenging enzymes catalase, Cu-Zn-superoxide dismutases and peroxidases [54]. The maintenance of H_2O_2 values near the control after TAs + LT suggest amendment of ROS homeostasis in pre-treated pea plants. In addition, it could be speculated that the increase found in carotenoids due to TA-14 + LT might also contribute to attenuation of ROS stress as carotenoids possess high capacity to scavenge ROS [55]. The decline in MDA content found after individual TAs application is consistent with the previous data for other pea cultivar (Ran 1) [23–25] and could be interpreted as lack of membrane damages or lack of oxidative stress. The application of TAs before LT treatment adjusts MDA values close to the control, which signifies for lessening of membrane damages and maintaining of the physiological redox state. These assumptions are in agreement with the lack of rise in H_2O_2 content after application of TAs prior to LT. In addition, it could be speculated that the increase in proline content found due to TAs plays the role of an antioxidant, which corroborate well with the observed reduction of H_2O_2 and MDA values in alone TAs and TAs + LT-treated plants.

5. Conclusions

The obtained data show that the LT treatment provoked typical stress-related physiological responses in pea. The alone TAs application had no effect on phenolics, but decreased stress markers while increased proline and leaf pigments. In the current study, we found that TAs decreased total polyamines, which was mainly on the account of conjugated PAs fractions. The TAs application prior to LT treatment diminished the alterations in stress biomarkers and free and bound PAs, and increased leaf pigments, conjugated Spd, and phenolics, which provided a better tolerance of pea plants to cold stress.

Author Contributions: Conceptualisation, J.J. and D.T.; methodology, S.J.; validation, I.S., D.T. and J.J.; formal analysis, D.T., R.M., J.J.; investigation, Z.K., E.J.-B.; data curation, D.T.; writing—original draft preparation, Z.K. and D.T.; writing—review and editing, I.S., S.J.; visualisation, I.S.; supervision, J.J.; project administration, E.J.-B.; funding acquisition, E.J.-B. All authors have read and agreed to the published version of the manuscript.

Funding: The study received financial support by project No 09.3.3-LMT-K-712-23-0166, funded by European Social Fund/European Regional Development Fund under grant agreement with the Research Council of Lithuania (LMTLT).

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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