The Influence of Impulse Pressure on the Phytohormone Content, Growth and Crop Productivity of Buckwheat Plants (*Fagopyrum esculentum Moench., cv. Aromat*)

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ABSTRACT

The foundation of plant productivity potential is laid at early phases of plant ontogenesis when germinal organs originate from meristems under the control of phytohormones which depend on the influence of external conditions, such as pressure. The influence of impulse pressure (IP) at 11-29 MPa for a duration of 15-20 μ sec on buckwheat seeds led to the germination reduction, but the mortality of seeds occurred not directly after the treatment but during the germination due to stress reactions. The lowering of germinating power depends on the intensity of IP. The IP treatment promotes the acceleration of cell division in root apical meristem and activation of morphogenesis in shoot apical meristem due to the increase in zeatin content in 8-day-old seedlings despite the growth of abscisic acid levels. The productivity of treated plants resulted in a 1.1 to 1.8-fold augmentation, mainly accounted for by an increase in the number of fruits.

(Key words: buckwheat, pressure, phytohormones, meristem, mitosis, germination, seedling, yield)

INTRODUCTION

Stress influences can be used to control growth processes in plants. Different kinds of artificial factors such as electromagnetic emanations and natural factors such as pressure, high or low temperature, etc. can affect plant ontogenesis and their potential productivity. Different factors affect numerous physiological processes simultaneously and so affect an ontogenesis as a whole. Small doses of stress factors activate some physiological processes. However, a lot of problems of plant response mechanisms have not yet been fully identified.

Pressure is an abiotic factor for plants. Sudden changes of osmotic pressure can promote some unspecific stress symptoms in plants, but normal osmotic pressure is an important factor for cell survival (Felix, Regenass, and Boller, 2000). The phloem transport is controlled by pressure gradients (Fensom et al., 1994). Pressure and mechanical forces are also factors of plant growth and development control (Dumais and Steele, 2000). We have proposed the method of pre-sowing seed treatment by impulse pressure (IP) generated by a shock wave (Atroschenko et al. 1997; Nefedieva, 2002). It has been shown that the pressures of 11, 23,

and 29 MPa promote accent changes in physiological processes of buckwheat plants (Nefedieva, Khryanin, 1999).

Phytohormones are known to play a role in plant protective responses. During stress reactions, the concentrations of phytohormones-inhibitors increase (Neumann et al. 1989, Takahashi and Suge, 1980) and the concentrations of phytohormones-activators decrease (Jackson et al. 1992). However, the majority of these findings are qualitative, while quantitative data on the relationship of the intensity of physical factors, phytohormone content, and the plant productivity are absent from the literature.

Plant productivity is an integrative process determined by a whole physiological complex. The foundation of plant productivity potential is laid at the early phases of plant ontogenesis. Root system, stem nodes, and leaves originate from meristems at the beginning of seed germination, inflorescence primordia are formed in seedlings. These parts formed under the control of phytohormones are responsible for future yield. Because the changes of phytohormone balance depends on the influence of external conditions, it is of interest to study the content of major phytohormones in plants treated by impulse pressure and their role in growth regulation. This is the aim of the research presented here.

MATERIALS AND METHODS

The subject of this research was buckwheat plants (*Fagopyrum esculentum Moench., cv.Aromat*). It is a middle-ripening cultivar zoned in the temperate regions. Plants were grown under phytotron and field conditions. Before sowing, seeds were treated by IP (Nefedieva, 2002; Atroschenko et al. 1997). There were up to 15,000 seeds treated at one time, and this mass was one of three biological series in each test.

The contents of endogenous phytohormones auxin (indole-3-acetic acid, IAA), gibberellic acid (gibberellic A_3 3-acetate, GA), zeatin ([trans-6-(4-hydroxy-3-methylbut-2-enyl)-aminopurine]), and abscisic acid (3-methyl-5-1-oxy-4-oxo-2,6,6-trimethyl-2-cyclogexen-1-il-2-cys,4-transpentadien acid, ABA) were determined at 1 day of soaking in seeds and at 8 days of germinating in leaves. The samples were fixed with liquid nitrogen and homogenized by grinding. Free phytohormones were extracted from homogenate three times for 24 hours each time with five volume of 80% aqueous ethanol. The combined extracts were filtered and concentrated to a water residue, at 40^{o}C and then were frozen, thawed and centrifuged at 800 g for 20 min.

The extracts were acidified with 1N HCl to pH 2.8 - 3.0. IAA, GA, and ABA were extracted from the aqueous solution with an equal volume of ethylacetate three times for 3 min each time. Then the extracts were dried, redissolved in ethanol and purified by thin layer chromatography (TLC) on Silufol UV-254 plates ("Kavalier", Czech) in isopropanol - 28% ammonia - water (10:1:1) solvent system. Individual substances were preparated from different zones. Solutions of IAA, GA, ABA ("Sigma", USA) were used as standards. The aqueous extracts were alcalinized to pH 8.0 - 9.0 with 1N NaOH and zeatin was extracted from the aqueous solution with an equal volume of water-saturated *n*-butanol three times for 3 min each time. Dried extracts were redissolved in ethanol and purified by TLC on the same plates in *n*-butanol - acetic acid - water (4:1:1) solvent system with the standard solution of zeatin ("Calbiochem", USA). Chromatographic eluates were dried, and a high performance

liquid chromatography (HPLC) analysis was performed using a Milikhrom-4 VUF ("Nauchpribor", Russia) equipped with a 2· 100 mm column with a 5 mm Nucleosil S16 sorbent. The parameters of the HPLC separation are shown in the Table 1. The substances were identified by their retention time, and their contents were computed with the WinChrom program.

Table 1. Parameters of HPLC.

Phytohormone	Parameters of HPLC			
	Solvent	Eluent	Rate of flow, $\mu l \cdot min^{-1}$	Wavelength, nm
IAA	Ethanol 96% aq.	Acetonitrile, 20% aq.	120	220
GA	Ethanol 20% aq.	Ethanol 20% in 2mM Tris-acetate buffer, pH 8,0	100	204
Zeatin	Methanol 20% aq.	Acetonitrile, 20% aq.	110	270
ABA	Methanol 40% aq.	Methanol 30% aq.	110	234

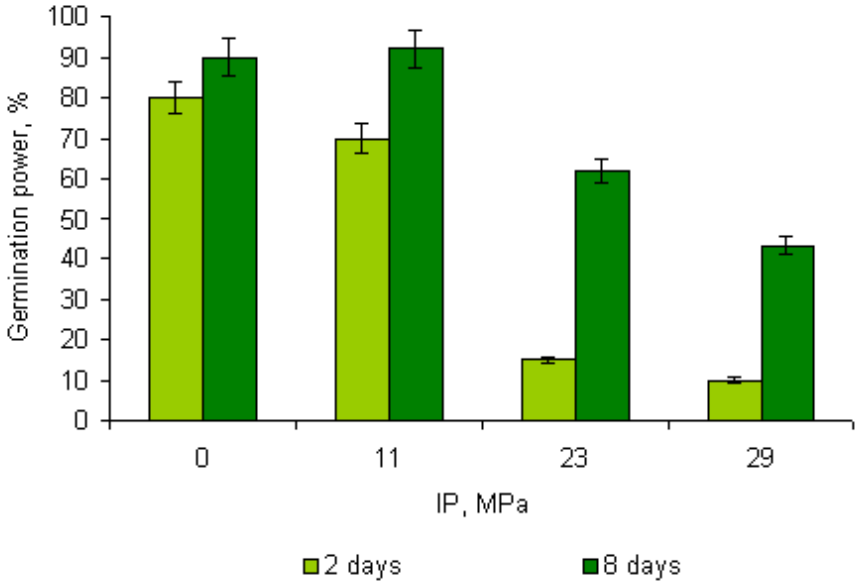
The germinating power was estimated by couching of seeds in a damp atmosphere at 25^{o}C. Germinated seeds were counted after 2 and 8 days. Mitotic index (MI) was determined in root apical meristems of seedlings aged 2 and 8 days. Material was fixed in acetic alcohol 6 times every 2 hours for 12 hours. Cell divisions are synchronized at this age, so we calculated the average MI from those 6 tests. Material was dyed in acetoorcein. Squashed preparations were analyzed with 600-times microscope magnification. MI was estimated as a percentage of M-phase cells to a gross amount of cells. Simultaneously we assessed for chromosome abnormalities by anaphase-telophase method.

The productivity of treated plants was examined in comparison with the control in three replicates (plots), 100 plants were sampled from the plot, and the number of fruits was determined on each plot. Fruits from each sample were combined, weighed, and the average fruit weight per plant was calculated. The weight of 1000 fruits was determined by the cross sampling technique. Calculated arithmetic means, standard errors of means, and Student-t criteria were used for the content of phytohormones. An ANOVA was used for calculation of plant productivity (Lane 2001).

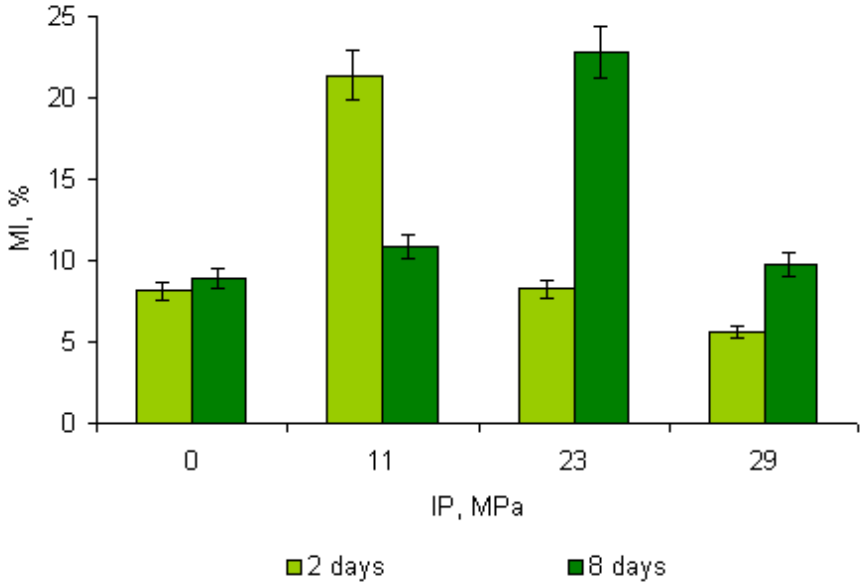
RESULTS AND DISCUSSION

Seed treatment by IP of different strength induced changes in physiological processes of plants at early phases of their development. It was found that the germinating power of seeds depends on the treated pressure (Figure 1, A). The decreased germinating power of 2-day-old seeds was caused the deceleration of physiological processes and the inhibition of root growth under the stress conditions. The germinating power of 8-day-old seedlings treated by IP 11 MPa, corresponded to the control level so this IP did not traumatize seeds significantly. Other IP strengths promoted the lowering of seed germination considerably (28-47%), but the amount of germinated seeds increased in tests with 8-day-old seedlings as compared to tests with 2-day-old seeds. The damage of seeds by pressure led to the germination reduction, but the mortality of seeds occurred not directly after the IP treatment but during germination due to stress reactions. The lowering of germinating power depends on the intensity of IP.

Figure 1. Germinating Power (A) and Mitotic Index (B) of Seeds Treated by Impulse Pressure.



a) Germinating Power



b) Miotic Index

Our investigations demonstrated that the average MI of control seedlings by the 2-3rd day was 8.1%, and it was 8.9% at 8-day-old seedlings, so MI was not changed (Figure 1, B). MI increased in seedlings treated by IP 11 MPa and formed 21.4% and 10.8% at the age of 2 and 8 days respectively, hence this pressure raised the intensity of mitosis in root apical meristems versus control. After treatment by IP 23 MPa, the average MI corresponded to control at 2 days (8.2%) and topped it by the 8th day (22.8%). IP of 29 MPa furthered the diminution of MI to 5.6% at 2 days, so the intensity of cell divisions decreased most significantly as compared to control. MI increased to 10.7% in 8-day-old seedlings. Accordingly we can see that there are two distinct phases in this analysis, such as the deceleration and activation of cell divisions. Despite the fact that IP of 11 MPa promotes early activation of MI (2 days), the seed germinating power was decreased. It is possible that the elongation of cells was inhibited. IP pressures of 23 and 29 MPa furthered the increase of MI in 8-day-old seedlings as compared to those at the age of 2 days. An IP of 29 MPa as compared to an IP 23 MPa served as an inhibitor of the growth and germination of seeds. Whereas chromosome aberrations were not observed in our tests the method of treatment by IP is recommended for widespread use.

The described growth processes depends on the endogenous phytohormone content (Table 2). IAA, zeatin, and GA activate plant growth, but their level decreased in our tests in 2-day-old seeds (except at 11 MPa, IAA). ABA content is known to increase under the effect of stresses (Neumann at al., 1989) and to inhibit the plant growth. Our tests showed the gain of ABA content in 2- and 8-day-old seeds/seedlings. The ratio of hormones showed the internal conditions of growth. The ratio IAA:ABA is the index of the possibility of cell elongation. This ratio was stable in control 2- and 8-day-old seeds/seedlings and differs in treated objects. IAA is known to stimulate the growth of fibers and the elongation of cells, so IP of 23 and 29 MPa inhibited the described processes. IP of 11 MPa promotes the significant increase of IAA and the ratio IAA:ABA in 2-day-old seeds, but high levels of IAA inhibit growth. The inhibition of

the growth of fibers and the elongation of cells was promoted by the phytohormone balance in 8-day-old seedlings treated by IP.

The ratio Zeatin:ABA demonstrated the conditions for the growth of shoots and cell divisions. It was decreased in 2 and 8-day-old seeds/seedlings. We can see that phytohormone balance inhibited these processes in germinating seeds and seedlings. A shift in the phytohormone ratio resulted from the increase of ABA content in treated seeds/seedlings. This hormone accumulated in 8-day-old seedlings as compares to 2-day-old seeds. However, there is evidence of the intensive growth of seedlings under similar conditions, and the protective role of ABA is shown (Wareing, Phillips, 1978). So the increase of the ratio IAA:Zeatin demonstrates the conditions for the cell elongation, and decrease of this ratio promotes the cell divisions. Despite the adversity for the cell divisions, the MI was increased in germinating seeds. So it was related to the damage of the cell division and the accumulation of cells in M-phase. Therefore the germination power was inhibited in seeds treated by IP. Favorable conditions for the cell divisions (decrease of the ratio IAA:Zeatin) were observed in 8-day-old seedlings. The increase of MI under these conditions was the evidence of real growth acceleration.

GA and ABA are terpene derivatives, therefore usually the changes of their concentrations are oppositely directed. The ratio GA:ABA decreased in 2- and 8-day-old seeds/seedlings because the IP induced the diminution of the content of GA. Gibberellins are responsible for promoting growth in the embryo of a seed. This ratio demonstrated the conditions for the growth of embryo and germination of seeds. The content of GA edged in germinating seeds (2 days) and lowered significantly in 8-day-old seedlings. There were probably, protective genetic sequences inhibited damage during germination. Our experiments demonstrated that phytohormones were involved in plant response to the effect of IP. Changes in the ratio between growth stimulators and inhibitors are the evidence of damage to the hormonal system, resulting in growth inhibition. The inhibition of vegetative growth activates plant development. The increase in zeatin content in 8-day-old seedlings was witnessed as compared to the control activated shoot morphogenetic programs.

Table 2. Content of Phytohormones in Germinating Seeds (2 days) and Leaves of Seedlings (8 days) after the Impulse Pressure Treatment (nmol· g $^{-}$ 1 of fresh weight).

Variant	Content of phytohormones, nmol · g $^{-1}$ fr wt				Ratio of phytohormones			
	IAA	Zeatin	GA	ABA	IAA: ABA	Zeatin: ABA	IAA: Zeatin	GA: ABA
2 days								
Control	21,3 ± 0,5	19,3 ± 0,8	163,3 ± 17,2	8,3 ± 0,2	2.6	2.3	1.1	19.7
11 MPa	34,9 ± 0,9*	11,1 ± 0,2*	132,9 ± 6,0	10,7 ± 0,1*	3.3	1.0	3.1	12.4
					1.5	1.2	1.3	11.8

23 MPa	15,9 ± 0,1*	12,4 ± 0,1*	122,7 ± 12,0	10,4 ± 0,1*				
29 MPa	17,9 ± 0,7*	5,7 ± 0,7*	81,7 ± 1,4*	8,8 ± 0,1*	2.0	0.6	3.1	9.3
8 days								
Control	432 ± 43	941± 88	611 ± 57	154 ± 15	2.8	6.1	0.5	4.0
11 MPa	408 ± 39	1261 ± 117*	389 ± 36*	570 ± 48*	0.7	2.2	0.3	0.7
23 MPa	306 ± 30	1086 ± 106*	862 ± 71*	589 ± 56*	0.5	1.8	0.3	1.5
29 MPa	414 ± 39	1038 ± 96	464 ± 47*	540 ± 47*	0.8	1.9	0.4	0.9

* Values significantly different from the control at $P = 0.5$. The means of 3 determinations and their standard errors are shown.

The influence of IP on seeds promoted the changes in shoot meristem activity of 8-day-old seedlings (Figure 2). The phase of germination is characterized by the forming of shoot apical meristem, germinal leaves, and their growth. Buckwheat stem apices transit to the reproductive stage after 3-4 leaf primordia and inflorescence primordia are formed. The increase of the diameter (11 MPa to 18%, 23 MPa to 9% and 29 MPa to 17%) and height (23 MPa to 16% and 29 MPa to 20%) of apices are shown in Figure.2. The result of the differentiation of apex was the formation of specialized tissues of germinal stem and primordia at the bottom of the meristem. Peculiarities of early ontogenetic phases was reflected in the morphogenesis of vegetative organs and flowers. The importance of this developmental stage consists in the formation of the assimilating apparatus and reproductive organs, both responsible for future yield. IP promoted the intensification of shoot growth processes.

Figure 2. Shoot Apical Meristems of 8-day-old Seedlings Treated by Impulse Pressure.

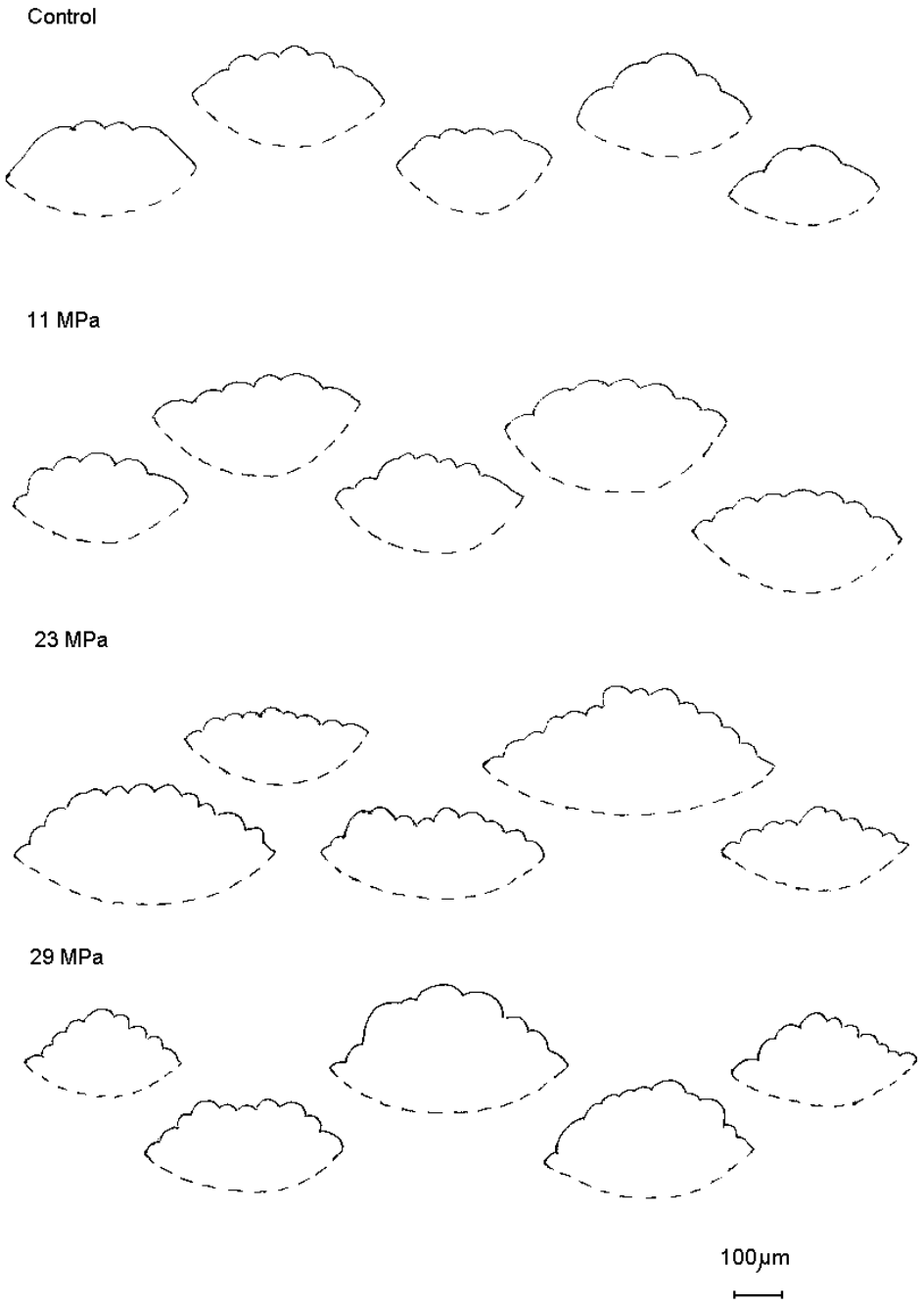


Table 3. Productivity of Buckwheat Plants after the Impulse Pressure Treatment.

Pressure, MPa	No. fruits		F	Total weight of 1000 fruits		F	Total fruit weight per plant,	
		%		g	%		g	%

0	58,2	100	753	25,55	100	304	1,48	100
11	100,4	173		26,74	105		2,68	181
23	88,2	152		25,18	98		2,22	150
29	64,1	110		25,29	99		1,62	109

*Values significantly different from the control at F_{01} = 5,6. The means of 3 determinations are shown.

The changes in plant productivity (Table 3) could be mainly accounted for by an increase in the number of fruits. It increased more significantly after the treatment of IP 11 MPa. It was the optimal stimulating dose of physical factor. The total weight of 1000 fruits did not change significantly. The total fruit weight per plant increased after the influence of IP reaching 1,8-fold maximum at 11 MPa. The development of the stress reaction resulted in the decrease of plant productivity after the influences of IP at 23 and 29 MPa as compared to 11 MPa.

Plant productivity is an integrative index by a whole complex of physiological process, namely, growth, metabolism and transport. It depends on the number of flowers and on the assimilating surface. Buckwheat plants are known to form numerous flowers, but normal fruits are developed only from 4-6% of them. Fruit abscision may be caused by infavorable external or internal conditions. In addition, the opening of numerous flowers provokes the abscision of them. The activation of growth and development in shoot apical meristems conduced the increase of flowers and provide favorable conditions for the supply of forming fruits with assimilates. The formation of assimilating surface and the supply of nutrients to the fruits seem to be essential for fruit formation.

CONCLUSIONS

The early stages of ontogenesis described here involved changes in the content of phytohormones. The damage of internal processes was observed in 2-day-old plants. Despite the increase of MI we can state the presence of growth inhibition during the germination of seeds. The level of plant growth inhibition, the peculiarities of the reparation of damage and the plant growth stimulation depend on the intensity of IP. The compensatory stimulation of growth and development in root and shoot apical meristems promotes the forming of internal tissues in roots and stems, the amplification of assimilating surface of leaves and the increase of the number and weight of fruits.

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