

# Sequential abiotic stress applied to juvenile eggplant modifies the seedlings parameters, plant ontogeny and yield

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## Abstract

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The main goal of this study was to evaluate eggplant (*Solanum melongena* L.) susceptibility to a sequence of stress factors during plant ontogeny based on seedling morphological, biochemical and physiological parameters and the subsequent assessment of yield characteristics. After germination, seedlings at the radical stage were exposed to chilling, heat, osmotic or oxidative stress. Four weeks after development in standard conditions in a greenhouse, the seedlings were again subjected to chilling stress in a growth chamber. A non-chilled Control was implemented. Then, the yield and physiological characteristics were assessed after field cultivation. Generally, stress application after germination resulted in better plant acclimation to chilling at the 4-week-old seedling phase, evaluated on the basis of photosynthetically active pigment contents, chlorophyll fluorescence and some morphological characteristics. The comparable time to reach successive phenological stages by stressed and Control plants in the field suggests that stress pretreatment does not retard eggplant development. These results also confirm the thesis that stress memory can be induced in eggplant by stressor application during the early stages of development.

**Keywords:** chilling; cross-tolerance; *Solanum melongena*; stress memory; yield

Eggplant (*Solanum melongena* L.) is one of the most important warm-climate vegetables in the world. The optimum temperature for seed germination is 24°C to 29°C, and for growth and fruit

development 21°C to 29°C (KÜRKLÜ et al. 1998), although SĘKARA (2010) showed that treating eggplant transplants with low temperature (up to 8°C) made it possible to control their morphology

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(height, weight, number of leaves, area of leaves, leaf area index, leaf area ratio) without a negative influence on subsequent plant development. There are few reports on the tolerance of vegetable species to stress factors applied in juvenile phases of development, especially during the stage of germination, when plant meristematic tissues show amplified sensitivity to stressors. The exposure to a particular stress that enhances the resistance to multiple other stresses is defined as cross-tolerance (CHEN, ARORA 2012). Plant metabolism can be altered by exposure to various stresses through sustained alterations in levels of key signalling metabolites. Alternatively, epigenetic changes could play a role by enabling long-term changes in gene expression responsible for memory stress (BRUCE et al. 2007; PASTOR et al. 2013). It is possible that memory stress, induced in the seedlings of tropical vegetables, can increase the complex tolerance to stress factors associated with field cultivation in temperate climatic zones. KOŚCIELNIAK and BIESAGA-KOŚCIELNIAK (2000) investigated the effect of short warm spells while chilling maize seedlings on water status, photosynthetic efficiency and final grain yield and stated that daily warming of plants at the seedling phase reduced the unfavourable effect of chilling on cob yield. On the other hand, CHINNUSAMY and ZHU (2009) determined that stress memory could have a negative impact on crop yield by preventing the plant from growing to its full potential. Thus, stress memory has implications regarding the use of seeds from stressed crops to raise subsequent crops by farmers, as well as breeding for stressful environments and *in situ* conservation of plant species.

So far, the possibility of eggplant treatment with controlled stress in a juvenile phase to induce cross-tolerance and stress memory has not been fully investigated. SĘKARA et al. (2012, 2015) described eggplant tolerance to stress factors in the seedling stage, and its prolonged effect on the chemical composition of seedlings and fruits. CHEN and ARORA (2012) assessed the phenomenon of increased tolerance to stress in germinating eggplant seedlings as the result of priming, but the cellular mechanisms behind this phenomenon have not been explained yet.

The aim of the present investigation was to evaluate eggplant susceptibility to stress factors during juvenile phase, on the basis of seedling morphological, biochemical and physiological parameters followed

by an assessment of yield characteristics. An investigation was made into the prolonged effect of stress factors applied at the seedling stage on phenological phases and selected yield parameters. The possibility of stress memory induction and maintenance during eggplant ontogenesis is discussed.

## MATERIAL AND METHODS

**Plant material and experimental set-up.** The experiment was conducted in 2011 and 2012 at the University of Agriculture in Krakow, Poland. Eggplant (*Solanum melongena* L.) cv. Epic F<sub>1</sub> seeds, sterilised with 50% thiuram (Organica-Azot, Jaworzno, Poland), were placed on Petri dishes (100 seeds per dish, in three replications of each below described object) with a layer of filter paper moistened with distilled water on March 11, 2011 and March 7, 2012. After germination in an incubator (3 days at 26°C), samples of 50 uniform seedlings from each Petri dish, at the radical stage, with radicles initially 0.5–1.0 mm long, were exposed to chilling (9°C for 48 h), heat (35°C for 2 h), osmotic stress (mannitol 0.2M for 2 h), oxidative stress (H<sub>2</sub>O<sub>2</sub> 0.2M for 2 h) or no stress (Control; 26°C for 48 h), according to the recommendations described by SĘKARA et al. (2012). Then, seedlings were placed in the standard peat substrate (in black 40-cell trays) and planted in a greenhouse with a day/night temperature of 20/17 ± 2°C. On April 14, 2011 and April 11, 2012, 4-week-old seedlings were subjected to chilling stress in a growth chamber. The temperature was gradually (1°C/hour) decreased to 8°C and maintained at this level for 7 days (relative humidity (RH) 60%, day-length 12 h, photosynthetic photon flux density (PPFD) about 500 μmol/m<sup>2</sup>/s). Control plants were divided in two; one group continued to grow in a greenhouse with a day/night temperature of 20/17 ± 2°C, while the second group was chilled as described above.

The six experimental treatments were as follows: (I) C – Control (26°C for 48 h) in the stage of 3-day-old seedlings, Control (20°C for 7 days) at the stage of 4-week-old seedlings; (II) C+Ch – Control (26°C for 48 h) at the stage of 3-day-old seedlings; chilling stress (8°C for 7 days) at the stage of 4-week-old seedlings; (III) Ch+Ch – chilling stress (9°C for 48 h) at the stage of 3-day-old seedlings; chilling stress (8°C for 7 days) at the stage of 4-week-old seedlings; (IV) H+Ch – heat stress (35°C for 2 h)

Table 1. Mean monthly temperature, photosynthetic active radiation (PAR) and the sum of rainfall in vegetation seasons 2011 and 2012

Month	2011			2012		
	temperature (°C)	PAR ( $\mu\text{mol}/\text{m}^2/\text{s}$ )	sum of rainfall (mm)	temperature (°C)	PAR ( $\mu\text{mol}/\text{m}^2/\text{s}$ )	sum of rainfall (mm)
May	14.1	721	54.6	15.6	692	21.4
June	18.6	613	41.0	17.8	621	106.0
July	17.9	340	163.0	20.3	437	42.8
August	19.4	419	37.2	18.7	363	46.4
September	15.5	307	14.4	14.1	241	30.6

at the stage of 3-day-old seedlings; chilling stress (8°C for 7 days) at the stage of 4-week-old seedlings; (V) Os+Ch – osmotic stress (mannitol 0.2M for 2 h) at the stage of 3-day-old seedlings; chilling stress (8°C for 7 days) at the stage of 4-week-old seedlings; (VI) Ox+Ch – oxidative stress ( $\text{H}_2\text{O}_2$  0.2M for 2 h) at the stage of 3-day-old seedlings; chilling stress (8°C for 7 days) at the stage of 4-week-old seedlings.

After the chilling treatment, the 4-week-old seedlings of each experimental group were divided into two groups. One group of seedlings was the material for analysis performed in three replicates, with 30 seedlings per replicate. The second group of seedlings was planted out in the field to analyse the phenological phases and the yield. This part of the experiment was also established in three replicates, with 10 plants per replicate. The experiment was established in split-block design, and experimental plots were surrounded by shelterbelts. Seedlings were planted in mid-May 2011 and 2012, at the Vegetable Experimental Station in Mydlniki, Poland (50°04'N, 19°51'E). The climate of the experimental station, located in southern Poland, is humid continental climate (Dfb) according to the Köppen's classification. The soil was classified as a Fluvic Cambisol (Humic) with respect to the FAO classification with a  $C_{\text{org}}$  level of 2% and  $\text{pH}_{\text{KCl}}$  6.11. The spacing was  $0.75 \times 0.6$  m (2.2 plants/m<sup>2</sup>). The amount of fertiliser was calculated on the basis of soil analyses to achieve a stable content of nutrients (mg/dm<sup>3</sup>): N – 100, P – 90, K – 220, Ca – 1,100, Mg – 70. Cultivation procedures (weeding, irrigation, plant protection against pests and diseases) were performed according to the standard recommendations for the species. Harvests were carried out from July to the end of September.

**Weather conditions.** Data concerning the mean air temperature, photosynthetically active radia-

tion (PAR) and sum of rainfall during the vegetation seasons in 2011–2012 are presented in Table 1. Data were collected from automatic HOBO Pro RH/Temp loggers to assess temperature and a HOBO Weather Station (Onset Comp. Corp., Cape Cod, USA) to assess light characteristics and rainfall at the experimental station. The analysis of the weather conditions showed that May and July of 2011 were characterised by lower temperatures but higher PAR values as compared to 2012. June, August and September were warmer in 2011 than 2012. The highest sum of rainfall was noted in July 2011 and June 2012.

**Morphology of 4-week-old seedlings.** After chilling, seedlings were subjected to biometrical measurements. The height of seedlings (measured from the base to the apex), the leaf surface area per plant (with the use of a program KS-RUN 3.0, Carl Zeiss Vision GmbH, Oberkochen, Germany), the leaf area index (LAI, defined as the ratio of plant leaf surface to the surface occupied by seedling) and the leaf area ratio (LAR, defined as the ratio of plant leaf surface to shoot dry weight) were determined. The fresh and dry weight of roots and shoots (after drying samples at 92–95°C until a constant weight was obtained) were recorded using a Sartorius A120S (Sartorius AG, Sartorius Lab Instruments GmbH & Co.KG, Göttingen, Germany) balance.

**Chlorophyll and carotenoid content; chlorophyll fluorescence of 4-week-old seedlings.** Chlorophyll *a*, chlorophyll *b* and carotenoid contents were determined by the modified LICHTENTHALER and WELLBURN (1983) method, after acetone extraction from 0.1 g fresh weight, collected from the first fully developed leaves of seedlings, at wavelengths of 646, 663 and 470 nm, respectively, with a Helios Beta spectrophotometer

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(Thermo Fisher Scientific Inc., Waltham, USA). Chlorophyll fluorescence parameters were measured at room temperature with a Handy-PEA fluorometer (Hansatech Instruments, Pentney, United Kingdom). One leaf (same age) was chosen per plant from each treatment. Before all measurements, leaves were dark-adapted for 30 min in a leaf clip, and then exposed to a saturation light pulse of 1 s duration and 3,000  $\mu\text{mol}/\text{m}^2/\text{s}$  intensity. The fast fluorescence kinetics (minimal  $F_0$  to maximal  $F_m$ ) were recorded to express the maximum quantum yield of photosystem II (PSII) according to equation  $F_v/F_m = (F_m - F_0)/F_m$ , where  $F_v$  was the yield of variable fluorescence,  $F_m$  – the maximal fluorescence of a dark-adapted sample, and  $F_0$  – the initial fluorescence value (MAXWELL, JOHNSON 2000). Plant vitality was also characterised by the performance index (PI), which reflects the functionality of PSI and II and provides quantitative information on the current state of plant performance under stress conditions (STRASSER et al. 2000).

**Phenological phases and yield characteristics.** The phenological phases were assessed for each experimental parameter. The number of days from sowing and transplanting to beginning of blooming (when more than 50% of plants had an open flower), as well as to the beginning of fruit setting (when more than 50% of plants had a fruit 1 cm in diameter), and to beginning and the end of harvest was counted. Fruits were harvested once a week, after reaching the size and colour sufficient for marketing, and divided into quality classes according to TRADE/WP.7/2000/11/Add.8, Dec. 2000. The early (first four harvests), marketable, and total yields were assessed.

**Statistical analyses.** For a synthetic description, the repeatable data were averaged over the two years of the experiment. The phenological phases and yield characteristics were presented separately for the two years of the experiment because of their dependence on meteorological conditions. Differences between means were evaluated with ANOVA, using Statistica Ver. 10 package (StatSoft Inc., Tulsa, USA), and the Tukey's HSD test was used to assess homogeneous groups at  $P = 0.05$ .

## RESULTS AND DISCUSSION

Plant plasticity is essential to overcome stresses that occur repeatedly during ontogeny, in order to

establish acclimation mechanisms that allow them to better respond at any time to single or multiple stresses of the same or different nature (MUNNÉ-BOSCH, ALEGRE 2013). In the present experiment, abiotic stress application during the germination of eggplant seeds induced changes that resulted in better acclimation to subsequent chilling at the 4-week-old seedling phase (Table 2). In contrast, significant decreases in shoot fresh weight, leaf area, LAI and LAR were found in plants that were not treated after germination and chilled at the phase of 4-week-old seedlings (C+Ch) as compared to the Control plants (C). Chilling pretreatment of seedlings and subsequent chilling (Ch+Ch) resulted in a significant decrease in the shoot fresh weight, leaf area, and the values of LAI and LAR as compared to C. Pretreatment with heat stress (H+Ch) did not cause any reduction in leaf area or in LAI and LAR values as compared to C. Shoots of plants not treated after germination and chilled only at the phase of 4-week-old seedlings (C+Ch) were significantly smaller than those pretreated with heat and osmotic stress before chilling (H+Ch and Os+Ch). The impact of these stressors was variable, but it confirmed the thesis that 'stress memory' and 'cross-tolerance' can be induced in eggplant following the application of stress factors in the early stages of development. VALLURU et al. (2012) specified morphological changes, i.e. a significant increase in leaf mass ratio, relative growth rate, a reduction in flag leaf size, total biomass and specific leaf area (SLA) as a result of early chilling of wheat.

The analysis of the pigment content and chlorophyll fluorescence parameters gave more obvious results (Table 3). The lowest content of chlorophylls was found in leaves of C+Ch seedlings. There was a significant, approximately 20% increase in the carotenoid concentration in leaves of Ch+Ch plants in relation to the leaves of plants in the remaining treatments. Carotenoids play a crucial role in photosystems and thylakoid membrane protection, so double chilling in the present experimental conditions may have caused the additional synthesis of these pigments. This dependence was confirmed by GIANNAKOULA et al. (2006) for lettuce treated at chilling temperature with high light intensity, which led to the accumulation of large amounts of carotenoids in the xanthophyll cycle in order to protect the plant against light damage. SZALONTAI et al. (2012) described the protective role of carotenoids in determining the stability of photo-

Table 2. Morphology and biomass of eggplant seedlings, as affected by stress factors application, means  $\pm$  SD for 2011–2012

Item	Treatment					
	C	C+Ch	Ch+Ch	H+Ch	Os+Ch	Ox+Ch
Shoot height (cm)	7.66 <sup>a-c</sup> $\pm$ 0.53	6.68 <sup>a</sup> $\pm$ 0.08	7.38 <sup>a-c</sup> $\pm$ 0.24	7.88 <sup>bc</sup> $\pm$ 0.23	8.33 <sup>c</sup> $\pm$ 0.26	7.08 <sup>ab</sup> $\pm$ 0.38
Root fresh weight (g/plant)	0.65 <sup>a</sup> $\pm$ 0.03	0.64 <sup>a</sup> $\pm$ 0.07	0.82 <sup>bc</sup> $\pm$ 0.16	0.76 <sup>ab</sup> $\pm$ 0.14	0.88 <sup>bc</sup> $\pm$ 0.12	0.92 <sup>c</sup> $\pm$ 0.12
Shoot fresh weight (g/plant)	2.94 <sup>d</sup> $\pm$ 0.10	2.02 <sup>a</sup> $\pm$ 0.26	2.61 <sup>b</sup> $\pm$ 0.33	2.64 <sup>bc</sup> $\pm$ 0.28	2.89 <sup>cd</sup> $\pm$ 0.15	2.82 <sup>b-d</sup> $\pm$ 0.17
Root dry weight (g/100 g f.w.)	8.35 <sup>a</sup> $\pm$ 0.62	9.75 <sup>bc</sup> $\pm$ 1.14	9.07 <sup>a-c</sup> $\pm$ 1.09	10.03 <sup>c</sup> $\pm$ 1.65	8.81 <sup>ab</sup> $\pm$ 0.45	9.45 <sup>a-c</sup> $\pm$ 0.75
Shoot dry weight (g/100 g f.w.)	9.93 <sup>a</sup> $\pm$ 0.30	12.95 <sup>b</sup> $\pm$ 2.51	10.53 <sup>a</sup> $\pm$ 0.70	10.41 <sup>a</sup> $\pm$ 0.94	10.20 <sup>a</sup> $\pm$ 0.68	10.27 <sup>a</sup> $\pm$ 0.71
Leaf area (cm <sup>2</sup> /plant)	95.2 <sup>d</sup> $\pm$ 8.11	58.6 <sup>a</sup> $\pm$ 8.04	71.6 <sup>bc</sup> $\pm$ 4.36	92.4 <sup>d</sup> $\pm$ 7.57	77.2 <sup>c</sup> $\pm$ 5.15	65.1 <sup>ab</sup> $\pm$ 6.34
LAI (cm <sup>2</sup> /cm)	3.81 <sup>d</sup> $\pm$ 0.33	2.35 <sup>a</sup> $\pm$ 0.32	2.87 <sup>bc</sup> $\pm$ 0.17	3.70 <sup>d</sup> $\pm$ 0.70	3.09 <sup>c</sup> $\pm$ 0.29	2.60 <sup>ab</sup> $\pm$ 0.41
LAR (cm <sup>2</sup> /g)	327 <sup>b</sup> $\pm$ 23.3	236 <sup>a</sup> $\pm$ 30.7	264 <sup>a</sup> $\pm$ 26.2	341 <sup>b</sup> $\pm$ 22.9	262 <sup>a</sup> $\pm$ 22.0	255 <sup>a</sup> $\pm$ 32.5

C – Control; C+Ch – Control at the stage of 3-day-old seedlings and chilling stress (8°C for 7 days) at the stage of 4-week-old seedlings; Ch+Ch – chilling stress (8°C for 48 h) at the stage of 3-day-old seedlings and chilling stress (8°C for 7 days) at the stage of 4-week-old seedlings; H+Ch – heat stress (35°C for 2 h) at the stage of 3-day-old seedlings and chilling stress (8°C for 7 days) at the stage of 4-week-old seedlings; Os+Ch – osmotic stress (mannitol 0.2M for 2 h) at the stage of 3-day-old seedlings and chilling stress (8°C for 7 days) at the stage of 4-week-old seedlings; Ox+Ch – oxidative stress (H<sub>2</sub>O<sub>2</sub> 0.2M for 2 h) at the stage of 3-day-old seedlings and chilling stress (8°C for 7 days) at the stage of 4-week-old seedlings; means in lines marked with the same letter for particular parameters do not differ significantly at  $P = 0.05$ ; SD – standard deviation

synthetic membranes under stress conditions. The higher values of the max. quantum yield of PSII ( $F_v/F_m$ ) and performance index (PI) were found in C plants as well as those pretreated with stressors before chilling than in C+Ch ones. A decrease of  $F_v/F_m$  below 0.830 is interpreted as an indicator for PSII photoinhibitory damage in response to stress, such as low temperature (GUIDI, DEGL'INNOCENTI 2012). The response of seedlings treated with chilling only once, at the 4-week-old seedling stage

(C+Ch), provided evidence of disturbed PSII activity, with a decrease in efficiency; this was confirmed by the measurements of PI for this treatment (2.64), which is considered a multi-parametric expression combining the three main functional steps within PSII – light energy absorption, excitation energy trapping and the conversion of excitation energy to electron transport (STRAUSS et al. 2006).

CHINNUSAMY and ZHU (2009) showed that stress memory may be inherited across mitotic or even

Table 3. Pigments' content and chlorophyll fluorescence parameters of eggplant seedlings leaves, as affected by stress factors application, means for 2011–2012

Item	Treatment					
	C	C+Ch	Ch+Ch	H+Ch	Os+Ch	Ox+Ch
Chlorophyll <i>a</i> (mg/100 g f.w.)	0.864 <sup>ab</sup> $\pm$ 0.139	0.725 <sup>a</sup> $\pm$ 0.141	1.115 <sup>b</sup> $\pm$ 0.159	0.985 <sup>ab</sup> $\pm$ 0.309	0.920 <sup>ab</sup> $\pm$ 0.202	0.910 <sup>ab</sup> $\pm$ 0.386
Chlorophyll <i>b</i> (mg/100 g f.w.)	0.397 <sup>ab</sup> $\pm$ 0.098	0.363 <sup>a</sup> $\pm$ 0.078	0.538 <sup>b</sup> $\pm$ 0.084	0.485 <sup>ab</sup> $\pm$ 0.161	0.456 <sup>ab</sup> $\pm$ 0.111	0.463 <sup>ab</sup> $\pm$ 0.386
Carotenoids (mg/100 g f.w.)	0.152 <sup>a</sup> $\pm$ 0.020	0.155 <sup>a</sup> $\pm$ 0.014	0.194 <sup>b</sup> $\pm$ 0.017	0.168 <sup>a</sup> $\pm$ 0.016	0.162 <sup>a</sup> $\pm$ 0.013	0.159 <sup>a</sup> $\pm$ 0.037
$F_v/F_m$ ratio	0.836 <sup>c</sup> $\pm$ 0.001	0.798 <sup>a</sup> $\pm$ 0.019	0.825 <sup>bc</sup> $\pm$ 0.011	0.829 <sup>bc</sup> $\pm$ 0.004	0.824 <sup>bc</sup> $\pm$ 0.004	0.816 <sup>b</sup> $\pm$ 0.005
PI index	6.57 <sup>b</sup> $\pm$ 0.512	2.64 <sup>a</sup> $\pm$ 0.357	5.47 <sup>b</sup> $\pm$ 0.557	5.74 <sup>b</sup> $\pm$ 0.718	4.96 <sup>b</sup> $\pm$ 0.470	4.45 <sup>ab</sup> $\pm$ 0.662

for abbreviations see Table 2;  $F_v$  – the yield of variable fluorescence,  $F_m$  – the maximal fluorescence of a dark-adapted sample; PI – performance index; f.w. – fresh weight

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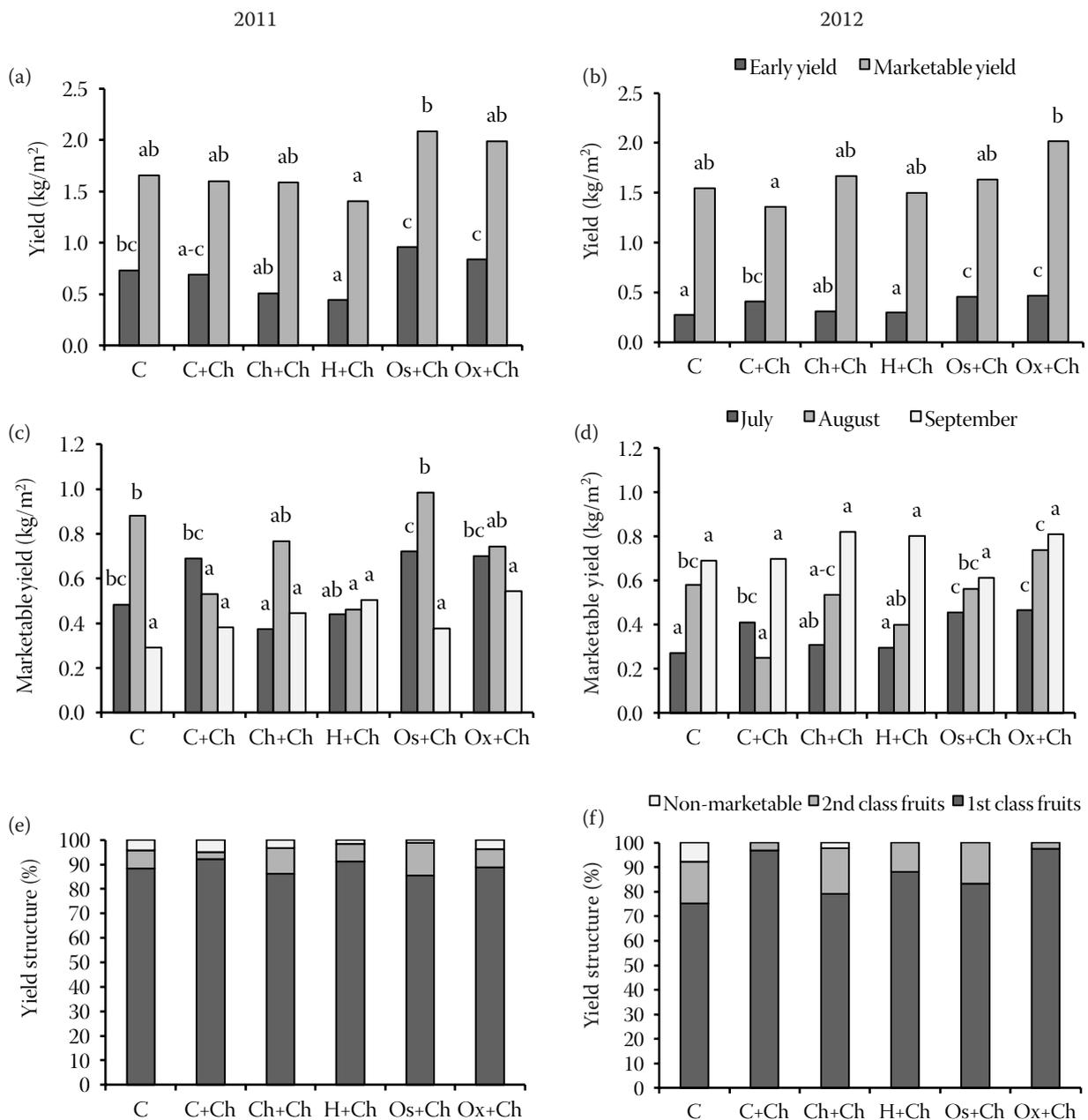


Fig. 1. Early and marketable yield (a,b), yield dynamics in harvest period (c,d) and yield structure (e,f) of eggplant fruits, as affected by stress factors application in a phase of transplants, in 2011 and 2012 for abbreviations see Table 2

meiotic cell divisions and may help plants cope more effectively with subsequent stresses during ontogenesis. Such 'reprogramming in phenology' was shown in our experiment, but the prolonged effect of stress factors applied at the seedling stage on the phenological phases of eggplant was minor (Table 4). In both years of the experiment, stress-pretreated specimens reached the stage of flowering 4 to 6 days later as compared to the Control.

However, at the beginning of fruit setting, these differences were smaller (0–3 days), and the first fruit harvests were performed on all plants at the same time, i.e. 57 (in 2011) or 50 (in 2012) days after transplanting. With planting time in mid-May, all harvests were completed at the end of September, before the first frost. The comparable time to reach subsequent developmental stages by stressed and Control plants suggests that the application of

Table 4. Phenological phases of eggplant treated with stress factors in a stage of seedlings

Transplanting	Days after transplanting											
	2011 (May 18)						2012 (May 16)					
	C	C+Ch	Ch+Ch	H+Ch	Os+Ch	Ox+Ch	C	C+Ch	Ch+Ch	H+Ch	Os+Ch	Ox+Ch
Beginning of flowering	27	31	31	30	30	30	27	33	33	33	29	29
Beginning of fruit setting	44	47	47	47	44	44	40	42	42	42	40	40
Beginning of harvest	57	57	57	57	57	57	50	50	50	50	50	50
End of harvest	134	134	134	134	134	134	135	135	135	135	135	135

for abbreviations see Table 2

an earlier sowing time than that applied in the present experiment, together with stress treatment, can cause a significant acceleration in plant development under field conditions, but this suggestion needs future investigations. Moreover, NOTO and MALFA (1986) found that the number of leaves preceding the first fruiting inflorescence decreased when tomato seedlings were exposed to 7°C at night for 2 weeks in comparison to 18–21°C. ILLANGAKOON et al. (2004) showed that days to 50% flowering and fruit number in eggplant are correlated with yield and can be used as indicators to predict the yield, but these parameters show a significant seasonal variability. UZUN (2006) showed that leaf number subtending the first fruit in tomato and eggplant declined linearly with decreasing temperature.

A study of the growth and development of eggplant under the field conditions in Poland contributed to the knowledge of the behaviour of this species in less favourable climatic conditions. Earlier studies by SEKARA (2010) showed the yield potential of the cv. Epic F<sub>1</sub> hybrid in field conditions in Poland, expressed by a mean marketable yield 3.34 kg/m<sup>2</sup>, including 22.5% early yield (first four harvests), but the vegetative and generative development of plants in the field depended mainly on microclimatic conditions. In both years, plants pretreated with stress factors gave a similar marketable yield of fruits compared to Control plants (Fig. 1). The yield dynamics in the harvest period was differentiated in experimental years and depended more on the meteorological conditions than the experimental treatment. The differences in the share of first and second class fruits in the yield were observed as the effect of stress pretreatment of seedlings. In both years of the investigation, the highest percentage of best quality fruits was harvested from C+Ch and Ox+Ch plants.

The chosen sequence of stress factors, especially osmotic or oxidative stress and then chilling (Os+Ch and Ox+Ch), caused an increase in early yield as compared to the Control. In the first year of the experiment, only a slight trend was observed, but in the second year, the differences were statistically significant. As these pretreatments triggered an increase in both the fresh and dry weight of roots, it is likely that this enabled developing plants to utilise water and nutrients more effectively. A study by VALLURU et al. (2012) demonstrated that exposure to early chilling stress during the seedling stage in two *Triticum* species resulted in adaptive responses, including early flowering, and alterations in several morphological and functional traits. MARTÍNEZ-ANDÚJAR et al. (2011) treated tomato seedlings with osmotic stress to improve tolerance to drought and salinity after transplanting. These authors suggested that during germination and early seedling development, plants sense and adapt to the growing conditions, which is important for stand establishment and crop yield. These mechanisms may facilitate plant acclimation to stress conditions and result from the retention of stress memory. Moreover, ALBACETE et al. (2006) succeeded at applying osmotic stress to tomato seedlings to enhance plant vigour during vegetation and fruit yield. They also stated that this method improved plant acclimation to the other adverse conditions such as salinity. It is in agreement with the present results, when different kinds of stressors were applied during germination, eggplant tolerance to chilling was modified in subsequent phases of development. The physiological mechanism of improved chilling sensitivity of eggplant, from the seedling stage to fruit formation, must be complex and is likely to comprise various alterations in plant metabolism which can be investigated in future studies.

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## CONCLUSION

The analysis of the chosen morphological parameters, the pigment composition and chlorophyll fluorescence of eggplant seedlings showed that stress application after germination resulted in better plant acclimation to chilling applied at the 4-week-old seedling phase in comparison to plants chilled only once, i.e. at the 4-week-old seedling phase only. The comparable time to reach subsequent developmental stages by stressed and Control plants grown in the field suggests that the application of an earlier sowing time together with stress application to germinated seeds can cause a significant acceleration in plant development under field conditions. Hence, extending the duration of the eggplant transplant stage when chilling is applied was suggested to accelerate plant development in the field in cool climatic zones, but this needs confirmation by future investigations. Moreover, the application of osmotic or oxidative stress during germination and then chilling was proposed to increase the early yield. The described procedures can also be adapted to other crop species to enhance plant vigour and stress tolerance. The thesis that stress memory can be induced in eggplant by stress factor application in the early stages of development can also be confirmed.

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