Response of wheat cultivars to salt stress by leaf chlorophyll fluorescence method

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Abstract. In laboratory experiments, the varietal specificity of the formation of adaptive reactions of 10-16 day-old seedlings of three varieties of spring wheat under chloride salinization (1.3%) according to the daily dynamics of chlorophyll fluorescence parameters (ChF) was established. The informative value of the ChF parameters Y(II), ETR, qP, Fv/Fm, Fv/Fo, Y(NPQ), qN and Y(NQ) as reliable biomarkers for early assessment of the resistance of varieties to chloride salinization at the level of photosynthetic apparatus has been confirmed. The reaction to chloride salinization of the more stable Sibirskaya 21 variety was less pronounced compared to the less stable varieties Novosibirskaya 29 and Novosibirskaya 41. The more stable variety has the smallest changes in the parameters of the ChF relative to the control. The greatest intervarietal differences in chloride salinization were manifested in 16-day-old seedlings. The level of photosynthetic activity in the formation of adaptive reactions during chloride salinization can serve as a criterion of stress resistance, which will speed up the selection of breeding material and increase its efficiency due to early rejection of unpromising samples.

1 Introduction

Soil salinity is one of the main limiting factors that negatively affect the growth and development of wheat in the main grain-growing areas of the world [1, 2]. In Russia, soil salinization accounts for about 20% of the area of agricultural land, and in some areas their share reaches 40-50% [3]. In the West Siberian region, the total area of saline soils is 8.8 million hectares, including arable land – 4.4 million hectares. The further expansion of the acreage of agricultural crops is also hindered by the secondary salinization of previously reclaimed arable land, the area of which is increasing [4]. The cultivation of grain crops on saline soils is accompanied by a significant decrease in productivity and deterioration of crop quality [5, 6].

One of the ways to overcome the negative effect of the salinity stressor may be the use of tolerant varieties, especially local breeding [7, 8]. Various screening methods are used to determine the salt resistance of genotypes. Salt resistance of varieties is compared in vegetation-field and greenhouse experiments. As a criterion of stability, the intensity of growth of aboveground parts and yield are used. In laboratory conditions, salt resistance is more often evaluated by the method of seedlings by changing the biomass and length of seedlings, the development of germinal roots, leaf area, and the water status of leaves [8]. To assess the salt resistance of plants, biophysical methods are also used – according to the output of electrolytes, spectral characteristics and changes in photosynthetic activity [9-11]. The advantages of the biophysical assessment of salt resistance include low labor intensity, sufficiently high information content, independence of diagnostic changes from environmental conditions, the ability to predict a decrease in the productivity of the variety at the early stages of plant development.

Photosynthesis is one of the stress-sensitive processes in a plant cell [12]. Violation of photosynthetic activity of plants can be assessed by the method of registration of chlorophyll fluorescence (ChF), which allows determining the overall bioenergetic status of a plant organism, that is, its ability to photosynthetic energy conversion [13]. The method is non-destructive, highly sensitive and allows obtaining information about the efficiency of photosynthesis and the integrity of the photosynthetic apparatus at the earliest stages of stress development [14]. The analysis of the fluorescence parameters ChF can be used as an accurate tool to check the reaction of unfavorable environmental conditions to photosynthesis and, consequently, to indirectly assess their impact on plants.

Salinity stress leads to a decrease in photosynthesis due to degradation of photosynthetic pigments, slowing down their synthesis and, as a consequence, to a significant change in the content and structure of leaf chlorophyll [15, 16]. The decrease in photosynthesis under salinization conditions is considered one of the most important factors limiting the growth and productivity of plants [17]. The study of the ChF kinetics can provide information about violations of the functioning of the photosynthetic apparatus of plants under the action of a salinity stressor [18, 19]. It has been shown that chloride salinization reduces photochemical efficiency by suppressing the activity of photosystem II (PS II) in wheat plants, more pronounced on the donor side than on the acceptor side of PS II [20]. In this case, the transfer of electrons from the primary acceptor of plastoquinone (Q A) to the secondary acceptor of plastoquinone (Q B) on the acceptor side of PS II is blocked, which leads to a decrease in the quantum yield of Fv/Fm photochemistry. According to Wani et.al a decrease in the quantum yield of PS II photochemistry is observed with an increase in the concentration of sodium chloride in the soil. At the same time, the occurrence of chlorophyll degradation in leaves coinciding with their aging is most pronounced in the sensitive genotype of mustard (Brassica juncea)[17]. An increase in the salt concentration in the soil of lettuce plants (Lactuca sativa L.) led to a decrease in the maximum quantum yield Fv/Fm, the quantum yield of unregulated energy dissipation Y(NO), an increase in the coefficient of non-photochemical quenching qN in PS II [21]. The effect of salinity stress on the indicators of Fo, Fm, Fv/Fm, and others in barley varieties is also shown [22]. However, there are reports of no effect of salinization on ChF [23]. It is noted that the effect of salt on the ChF parameters is mainly observed with a high salt content in the medium, and the reaction to salinity stress depends on the genotype [18]. The paper [24] shows the possibility of using a non-invasive fluorometric method using PAM technology to assess damage to various plants under salinity stress.

Currently, science and practice have mainly established an appropriate set of salt-resistant species for various agro-climatic zones. At the varietal level, a small number of such studies have been conducted, and there is insufficient data in the literature on the study of salt resistance of varieties of agricultural crops, including spring soft wheat.

The aim of the work is to investigate the adaptive reactions of spring wheat varieties under chloride salinization by changing the parameters of the ChF kinetics to identify the relationship of indicators with the stability of varieties.

2 Conditions, materials and methods

The experimental work was carried out at the Siberian Institute of Physics and Technology of the Siberian Branch of the Russian Academy of Sciences in the Laboratory of Physical Processes in Agrophytocenoses.

The research was carried out in greenhouse experiments (solution cultures) in laboratory conditions on seedlings of recognized varieties of middle-early spring soft wheat: Novosibirskaya 41, Sibirskaya 21 and Novosibirskaya 29 of the selection of the Siberian Research Institute of Plant Growing and Breeding – Institute of Cytology and Genetics of the Siberian Branch of the Russian Academy of Sciences.

Experiment variants:

a) control (without stress load);

b) chloride salinization (1.3% NaCl).

The level of stress load – 1.3% NaCl – was determined by us in specially conducted greenhouse experiments as allowing us to differentiate Siberian wheat varieties when assessing their resistance to this stress factor by biometric indicators and permeability of cell membranes [25].

Wheat seeds were pre-sterilized with 96% ethyl alcohol for 1 min, followed by three times rinsing with distilled water. Then the seeds were placed in Petri dishes with moistened filter paper and germinated in a thermostat at a temperature of 22 °C for three days.

Next, the seedlings were grown in the Biotron-7 clima camera in a roll culture on tap water at a day–night photoperiod of 16 and 8 hours, respectively, illumination of 20,000 and 0 lux (day – night), temperature of 22 °C and 18 °C (day – night), humidity of 60%.

The ChF daily kinetics and parameters were recorded using a Dual-PAM-100/F fluorimeter (Heinz Walz, Germany) using the pulse-amplitude modulation method in the recording mode of the slow kinetics of dark induction curves with pulse saturation analysis. Before the fluorescence measurement, 10-16 day-old wheat seedlings were adapted to the darkness in the sample chamber for 30 min. The following fluorescence parameters were obtained: Fo, Fm – the minimum and maximum ChF level caused by a pulse of light after the adaptation of leaves to darkness; Fo', Fm' – the minimum and maximum ChF level caused by a pulse of light after the adaptation of leaves to light; Fv /Fm – the maximum photochemical quantum yield of PS II; Y(II) – effective photochemical quantum yield of PS II after adaptation of leaves to light; Y(NPQ) – quantum yield of regulated non-photochemical quenching of ChF; Y(NO) – quantum yield of unregulated non-photochemical quenching of ChF; qP - coefficient of photochemical quenching of ChF; qN - coefficient of non-photochemical quenching of ChF; ETR – electron transport rate. The variable ChF was calculated: Fv = Fm – Fo and Fv / Fo – a value proportional to the activity of the water-splitting complex on the donor side of PS II.

The reaction of the variety was determined by the relative change in the measured parameters of the ChF of seedlings after exposure of plants to a stressor. The smaller the parameter changes, the higher the stability in the studied group of varieties. The repetitions of the analytical and biological experiments are 6 and 3-fold. Statistical data processing was carried out in Microsoft Excel 2010 using a standard data analysis package. The ChF parameters recorded within 4 minutes were analyzed. The average error did not exceed 1.5-2.0%. Three series of experiments were conducted. To determine the significance of the differences in the mean values, the Student's t-test was used.

3 Results and discussions

The studies were carried out to confirm the possibility of using the method of registering the ChF parameters to study the formation of adaptive reactions of seedlings of three wheat varieties depending on their age during cultivation and to assess stress resistance to chloride salinization. The PAM measurements of ChF used in this study generate various parameters, which are mainly derived from five mutually independent levels of ChF: minimum (background) Fo and maximum fluorescence outputs Fm in a dark-adapted state; stationary Fs; minimum (background) Fo' and maximum fluorescence outputs Fm' in the light-adapted state of the samples, respectively.

The level of tolerance of varieties to salinization was determined by their reaction to a salinity stressor during the growth and development of seedlings. Our research has established the primary manifestation of stress reactions, as an increase in the thermal scattering of light energy in seedlings of 10-day-old age. The parameter of controlled non-photochemical quenching – the quantum yield Y(NPQ) significantly ($p \leq 0.05$) increased in all varieties in the range of 14.9–21.5% with an unreliable change in the remaining parameters compared to the control. On the 12th day of cultivation, a significant decrease in the parameters of photochemistry was found – the effective photochemical quantum yield Y(II) and the electron transport rate ETR only in seedlings of the Novosibirskaya 29 variety by 17.8 and 18.9%, respectively. In the Sibirskaya 21 variety, the values of only the parameters F_V and F_V/F_0 increased by 22.7 and 36.1%.

Significant disturbances in the intensity of photosynthesis associated with the toxic effect of chloride salinization were recorded in 14-day-old wheat seedlings (table).

Table 1. Daily dynamics of chlorophyll fluorescence parameters in the leaves of seedlings of wheat cultivars under chloride salinization.

Seedling age, days	Option	Parameters							
		Y(II)	Y(NPQ)	Y(NO)	qP	qN	ETR	F_V/F_m	F_V/F_0
		cultivar Novosibirskaya 41							
14	control	5.9	0.9	5.9	3.5	2.3	241.3	0.68	2.1
	NaCl	4.1*	1.6*	6.3*	3.2	3.1*	163.1*	0.66	2.0
16	control	6.1	0.9	4.9	3.8	2.4	248.9	0.65	1.8
	NaCl	2.7*	1.9**	7.4*	2.5*	3.4*	102.6*	0.51	1.0*
		cultivar Novosibirskaya 29							
14	control	5.9	0.85	4.3	3.6	2.1	226.6	0.72	2.5
	NaCl	4.7*	1.14*	6.1*	3.1*	2.4	189,2*	0.72	2.5
16	control	5.2	1.19	5.5	2.9	2.6	210.5	0.74	2.7
	NaCl	2.4*	1.70*	7.8*	2.2*	2.8	92.2*	0.62*	1.6*
		cultivar Sibirskaya 21							
14	control	6.4	0.76	4.7	3.5	2.1	263.5	0.71	2.4
	NaCl	5.6*	1.18*	5.1	3.5	2.8*	229.4*	0.69	2.3
16	control	6.4	1.11	4.5	3.6	2.9	260.6	0.70	2.4
	NaCl	4.7*	1.22	6.0*	3.3	2.6	190.5*	0.67	2.0*

Note: * - the differences with the control are significant at the 5% significance level.

At the same time, the inhibitory effect of chloride salinization on photochemical activity in all varieties was found in the range from 12.2 to 32.4% ($p \leq 0.05$) compared with the control. The most significant ($p \leq 0.05$) synchronous decrease in Y(II) and ETR was observed in the Novosibirskaya variety 41 – 30.5 and 32.4%, respectively. The toxic effect of chloride salinization led to an increase in the thermal dissipation of the energy of excited chlorophyll – the values of the parameters of controlled photochemical quenching Y(NPQ) and qN significantly ($p \leq 0.05$) increased in all varieties in the range of 32.1–81.8%. The parameter of unregulated non-photochemical quenching Y(NQ) associated with the generation of free radical oxidation significantly ($p \leq 0.05$) increased in varieties Novosibirskaya 41 and Novosibirskaya 29 by 21.0 and 40.1% compared with the control.

On the 16th day of cultivation, under conditions of increasing destructive action of chloride salinization, a significant ($p \leq 0.05$) decrease in the effective quantum yield Y(II), the electron transport rate ETR was found in all varieties from 25.5% (Sibirskaya variety 21) to 56.5% (Novosibirskaya variety 41) compared with the control. The qP parameter showing the share of light energy consumed by the "open" RC PS II (before the application of the saturating light flash) decreased to a greater extent in the varieties Novosibirskaya 29 and Novosibirskaya 41 (up to 27.1 and 33.2%), respectively, compared with the control. The seedlings of the Sibirskaya 21 variety showed unreliable changes in qP compared to the control.

The inhibition of light-dependent reactions was accompanied by a significant ($p \leq 0.05$) increase in the values of the parameters of non-photochemical quenching of the ChF coefficient qN, quantum yields Y(NQ) and Y(NPQ*)* from 42.9% to 115.4% in the variety Novosibirskaya 41. The Novosibirskaya 29 variety significantly increased the Y(NQ) and Y(NPQ) parameters by 41.1 and 42.9%, and the Sibirskaya 21 variety showed a significant increase only in the Y(NQ) parameter to 34.1% compared to the control.

Thus, in general, under conditions of chloride salinization, there was an increase in photochemistry inhibition from 30.5 to 56.5% and an increase in thermal energy dissipation of excited chlorophyll PS II from 40.1% to 115.4% in the leaves of wheat seedlings from 14 to 16 days of cultivation in all varieties.

The variety specificity of the formation of adaptive reactions under salinity stress, depending on the age of seedlings, has been established.

In the Novosibirskaya 41 variety, photosynthetic activity was suppressed from the 14th to the 16th day of cultivation, which led to a significant ($p \leq 0.05$) decrease in the values of the parameters Y(II), ETR, qP by 1.9, 1.8 and 3.5 times, respectively. Compared with 14 days, on day 16 there was an increase in the parameters Y(NPQ), Y(NQ) and qN by 1.4, 2.3 and 1.2 times. A significant ($p \leq 0.05$) increase in the parameters of non-photochemical quenching of ChF - Y(NPQ), Y(NQ) and qN was 115.4, 48.1 and 42.9%, respectively, compared with the control. In addition, inhibition of Fm, Fv, Fv/Fo was found by 32.8, 47.1 and 44.0% compared to the control, exceeding the values of the parameters of the Sibirskaya 21 and Novosibirskaya 29 varieties.

The nature of the formation of adaptive reactions during salinization of the medium in the Novosibirskaya 29 variety is similar to the Novosibirskaya 41 variety, but differs in the amplitude of changes in the ChF parameters. A significant ($p \leq 0.05$) increase in the parameters of non-photochemical quenching of ChF - Y(NPQ) and Y(NQ) by 42.9 and 41.1%, respectively, compared with the control was found. The qN parameter was not changed reliably. The inhibition of Fm, Fv, Fv/Fo and Fv/Fm was found to be 12.8, 26.7, 41.8 and 16.0% compared to the control. The Fo parameter significantly increased by 26.0%. Compared with 14 days, on day 16 there was an increase in Y(NPQ) and Fo by 1.3 and 1.9 times. The values of the parameters Y(NQ) and qN changed unreliably.

Adaptation of Sibirskaya 21 seedlings to salinization is associated with a decrease in Y(NPQ) and qN by 5.0 and 3.0 times, respectively. However, there was a 4.1-fold increase in the parameter Y(NQ) on day 16 compared to the value of the parameter on day 14 of seedling cultivation, which leads to blocking of electron transfer along the electron transport chain and a 2.0-fold decrease in the photochemistry parameters Y(II) and ETR. The Fv/Fo parameter decreased to 14.1% compared to the control. The parameters Fo, Fm, Fv, Fv/Fo changed unreliably compared to the control.

There are two main target sites for stressors in chloroplasts: the electron transport chain (ETC) and the synthesis of chlorophyll and carotenoids. ETC with its electron carriers and enzymes participates in the phosphorylation and photo-recovery of NADP, whereas the synthesis of chlorophyll and carotenoids can be associated with the light-harvesting complex

(LHC) and the antennas of photosynthetic reaction centers [26]. By registering the ChF parameters, you can detect changes in these target sites.

The difference in the change in the parameters of photosynthetic activity under the same stress load of chloride salinization between the varieties Novosibirskaya 29, Sibirskaya 21 and Novosibirskaya 41 indicates different tolerance mechanisms and strategies for converting light energy into chemical energy during photosynthesis. In general, plants respond to stressors by activating protective and adaptive mechanisms in order to maintain photosynthetic activity to adapt to new environmental conditions. This may include an increase in the ability to dissipate energy, which is detected by increasing the parameters of non-photochemical quenching Y(NPQ) and qN without changing the maximum quantum efficiency of PS II Fv/F_0 and Fv/Fm. [27]. We observe such a process in seedlings of all varieties on the 10th-12th days of cultivation. At the same time, partial photoinhibition occurs in the Novosibirskaya 29 variety, detected by a decrease in the effective photochemical quantum yield Y(II) and the electron transport rate ETR.

The increasing stress stimulation on the 14th and 16th days of seedling cultivation led to a decrease in the effective quantum yield *Y*(II), the photochemical quenching coefficient qP and the electron transport rate ETR in all varieties*,* which means inhibition of the electron transport chain and suppression of photosynthetic activity. The inhibition of light-dependent reactions was accompanied by an increase in Y(NPQ), Y(NQ) and qN compared to the control in all varieties. A decrease in the values of the ChF variable Fv parameter indicates a weakening of photosynthetic activity and damage to thylakoids, and a decrease in background Fo and maximum Fm ChF indicates a negative effect of chloride salinization on the antenna complex, leading to a loss of energy during its migration [14]. However, no irreversible loss of PS II functionality was observed in our experiment. According to Pérez-Bueno et al, only in the case of a serious irreversible loss of the functionality of PS II, the parameters of photochemical and non-photochemical quenching of ChF can decrease [27].

The selected stress load of sodium chloride (1.3%) and the duration of its action (up to 16 days of cultivation) under experimental conditions allowed us to establish intervarietal differences. The greatest intervarietal differences (up to 52.6%) under the action of salinization were observed on the 16th day of seedling cultivation. On the 14th day of cultivation, the intervarietal differences amounted to 30.6%. As a result of the ranking, the resistance of varieties to chloride salinization varied in descending order: Sibirskaya 21 – Novosibirskaya 29 – Novosibirskaya 41. The protective systems of the Sibirskaya 21 variety had greater lability (a decrease in Y(II) and ETR by 25.5 and 26.9%, unreliable changes in Y(NPQ), qN, Fo, Fm, Fv, Fv/ Fo compared with the control). The greatest destructive effect of chloride salinization was found in the Novosibirskaya 41 variety (decrease in Y(II) and ETR by 56.5 and 58.8%, decrease in Fm, Fv, Fv/Fo by 32.8, 47.1 and 44.0%, increase in Y(NPQ), Y(NQ) and qN by 115.4, 48.1 and 42.9 % compared with the control (Figures 1-3).

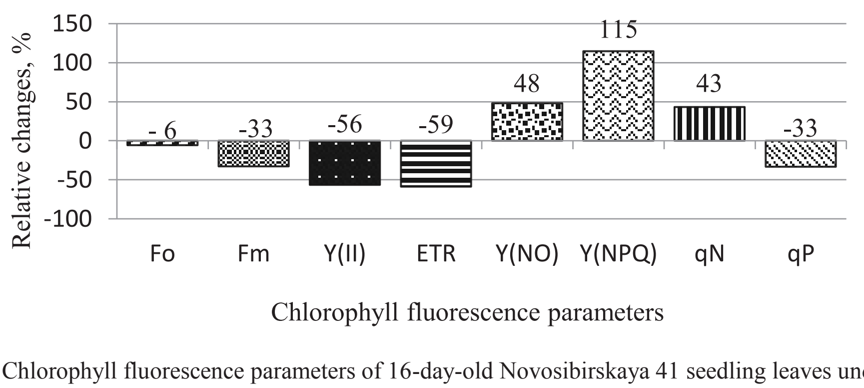

Fig. 1. Chlorophyll fluorescence parameters of 16-day-old Novosibirskaya 41 seedling leaves under chloride salinization (relatively unresistant).

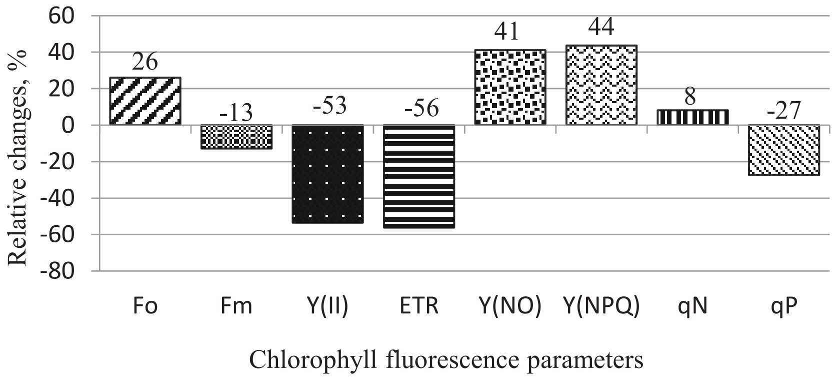

Fig. 2. Chlorophyll fluorescence parameters of 16-day-old Novosibirskaya 29 seedling leaves under chloride salinization (relatively unresistant).

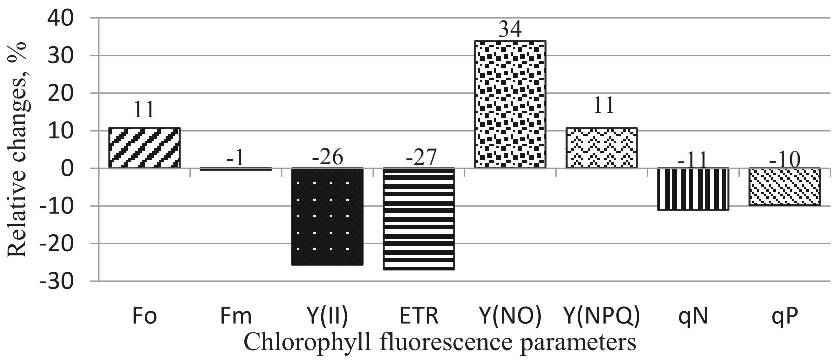

Fig. 3. Chlorophyll fluorescence parameters of 16-day-old Sibirskaya 21 seedling leaves under chloride salinization (relatively resistant).

It should be noted that the reaction of the Sibirskaya 21 variety, which is more resistant to chloride salinization, turned out to be less pronounced – the smallest or unreliable changes in the ChF parameters relative to the control compared to the more sensitive varieties Novosibirskaya 29 and Novosibirskaya 41. Quamruzzaman et al. also established the destructive effect of salinization on the photochemistry of leaves by a significant decrease in the values of the Fv/Fm ratio in sensitive wheat genotypes compared to less sensitive ones [6]. In a study by Wani et al. the maximum decrease in the Fv/Fm parameter was recorded in the more sensitive *Brassica juncea* variety at different levels of chloride salinity [17]. The results of the study by Saddiq et al. made it possible to identify stable genotypes of *Triticum aestivum* L. having higher values of the parameters Fv'/Fm' and Ft under salinization conditions [24]. The identified genotypes can be a valuable resource for genetic improvement programs that allow for a better understanding of plant resistance to salinity stress.

4 Conclusion

The conducted studies confirm the informative value of the ChF parameters Y(II), ETR, qP, Fv/Fm, Fv/Fo, Y(NPQ), qN and Y(NQ) as reliable biomarkers for assessing the resistance of varieties of soft spring wheat to chloride salinization at the level of photosynthetic apparatus. The parameters adapted to light were more sensitive than the parameters adapted to darkness. Thus, the former may be preferred in stress tests. The Fv/Fm parameter, which is most often reported in the literature, turned out to be the least sensitive parameter compared to its analogue Fv/Fo. The age of seedlings (14-16 days) and the concentration of sodium chloride (1.3%) were established to differentiate varieties by stability. The level of photosynthetic activity in the formation of adaptive reactions during chloride salinization can serve as a criterion of stress resistance, which will speed up the selection of breeding material and increase its efficiency due to early rejection of unpromising samples. The ChF registration method is non-destructive and reduces labor costs, demand and loss of plants.

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