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DROUGHT INDUCED DAMAGES OF PHOTOSYNTHESIS IN BEAN AND PLANTAIN PLANTS ANALYZED *IN VIVO* BY CHLOROPHYLL *A* FLUORESCENCE

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Abstract

KRASTEVA, V., V. ALEXANDROV, M. CHEPISHEVA, S. DAMBOV, D. STEFANOV, I. YORDANOV and V. GOLTSEV, 2013. Drought induced damages of photosynthesis in bean and plantain plants analyzed *in vivo* by chlorophyll *a* fluorescence. *Bulg. J. Agric. Sci.,* Supplement 2, 19: 39–44

Plant uses complex responses to protect against drought, including changes in gene expression, cell metabolism and whole plant physiology. Drought affects photosynthetic apparatus at different levels of organization. Using of new developed techniques for simultaneous registration of prompt and delayed fluorescence, give opportunity to achieve substantial progress in investigation of the drought effects on photosynthesis in intact leaves. The stress reaction of photosynthetic machinery is monitored in leaves of whole plants of *Phaseolus vulgaris* and *Plantago major* during 12-days cessation of irrigation. In opposite to drought sensitive bean plants subjected to 12-day drought treatment, photosynthetic apparatus of plantain reveals earlier response to increasing water deficit. It was expressed by a visible decrease in fluorescence yield and suppression of photosynthetic efficiency. The shape of delayed fluorescence induction is found to be the most sensitive indicator for changes in photosynthetic electron transport in plants subjected to drought stress. As a parameter for estimation of drought stress effects could be considered the second maximum of sub-millisecond delayed fluorescence induction curve, while both microsecond and millisecond delayed fluorescence components did not change significantly. The changes of the second delayed fluorescence induction maximum could be interpreted as decreased rate of intersystem electron flow in tolerant *Plantago* plants. This suggestion is also supported by significant decrease in the rate of $P700^+$ reduction that was monitored by light reflection at 820 nm. We proposed that intersystem electron transport suppression could be related to adjustment of electron transport chain for following switch to cyclic electron flow around Photosystem I to secure photosynthetic electron transport with protection against drought-induced damages.

Key words: chlorophyll *a* fluorescence, delayed fluorescence, drought stress

Abbreviations: PF – prompt fluorescence; DF – delayed fluorescence; F_m – maximal prompt fluorescence; $DF_{0.1}$ – DF measurements during dark intervals of 0.02–0.09 ms; $DF_{1.0}$ – DF measurements were registered during dark intervals of 0.1–0.9 ms; $DF_{2.3}$ – DF measurements were registered during dark intervals of 1.0 ms to 2.3 ms; MR_{820} – modulated 820 nm reflection; P700 – the first electron donor in PS I reaction center; RWC – relative water content; A_{pot} photosynthetic metabolic potential; Multifunctional Plant Efficiency Analyser – M-PEA; Q_A and Q_B – the first and second quinone acceptors in PSII; PQ – plastoquinone.

Introduction

Plant and crop productivity closely depends on complex interacting environmental factors during their vegetation and by genetically determined tolerance against unfavorable environment (Baker, 1997). For effective growth of the crops, it is necessary to know intimate mechanisms of the responses of crops to stress.

Among other stresses drought has appeared as one of the most deleterious factors inducing great decrease

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in crop yields. Plant uses complex responses to protect against drought including changes in gene expression, cell metabolism and whole plant physiology.

One of the most commonly experienced stress conditions in nature is the occurrence of mild drought stress, i.e., RWC 70%, (Loreto et al., 2004). It is generally accepted that plant growth is affected well before any effects on photosynthesis can be observed (Nonami and Boyer, 1990). The inhibition of photosynthesis in drought-stressed leaves is often relieved when these leaves are exposed to very high CO_2 concentration (e.g., Cornic et al., 1989) It is widely held that the photosynthetic apparatus is resistant to drought (Cornic and Massacci, 1996). However, when RWC falls below 70%, a cascade of physiological processes can be affected resulting in irreversible or slowly reversible damage to the photosynthetic apparatus (e.g., Kaiser, 1987; Cornic and Massacci, 1996).

Drought decreases photosynthetic rate *via* decreased stomatal conductance for CO_2, and photosynthetic metabolic potential, A_{pot} (Lawlor and Tezara, 2009). Photosynthetic rate decreases as stomatal conductance falls. At a given intercellular CO_2 concentration photosynthetic rate decreases, showing impaired metabolism. The intercellular and probably chloroplast CO_2 concentration, decreases and then increases. Photosystem activity is unaffected until very severe drought stress (Lawlor and Tezara, 2009). In intact leaves, the electron transport between the two photosystems is decreased in favor of Fd-dependent cyclic electron flow at reduced concentrations of oxygen and carbon dioxide (Bukhov and Carpentier, 2004). In nature, such situation is often observed under drought stress, which causes stomata closure (Wu et al., 1991). This provides preferential involvement of cyclic electron transport to the energization of the thylakoid membrane and, consequently, to the control of PSII photochemistry (Katona et al., 1992). Low photosynthetic rate, together with photorespiration, which is maintained or decreased, provides a smaller sink for electron transport, causing overenergization of energy transduction.

Because photosynthetic apparatus could be considered as resistant to drought (Cornic and Massacci, 1996) it is difficult to find closely related to drought changes in intact plants that could be used as markers for rapid tests for damages in plants in the field. Using newly developed techniques for simultaneous registration of prompt and delayed fluorescence, gives us opportunity to achieve substantial progress in investigation of the drought effects on photosynthesis in intact leaves (Goltsev et al., 2009; Strasser et al., 2010). In this investigation was found that photosynthetic electron transport is inhibited on the level of intersystem chain between PSI and II in drought tolerant plants and this inhibition should be considered as down regulation rather than damages.

Material and Methods

In present investigation, two kinds of plants – drought sensitive bean plants and drought tolerant plantain plants – were used. Bean plants (*Phaseolus vulgaris* L. cv. Cheren Starozagorski) were grown on fertilized soil mixture in a chamber at controlled temperature (22–23ºC), light (200 μmol photons m^{-2} s^{-1}), and humidity (40–50%) at photoperiod 12/12 h. 10 days old plants were decapitated by removing the stem, buds, apex and the newly appearing leaves just above the already formed leaves as in Yordanov et al. (2008). Primary leaves of 20–25 days old decapitated plants were used in the experiments.

Plantain (*Plantago major* L.) plants were harvested from their natural habitats and transferred to pots with soil – peat mixture and were grown at natural light.

The kinetics of prompt chlorophyll *a* fluorescence (PF), delayed Chl a fluorescence (DF) and modulated reflection at 820 nm (MR) were simultaneously recorded by the Multifunctional Plant Efficiency Analyzer, M-PEA (built by Hansatech Instrument Ltd., UK). Measurements were conducted on dark-adapted (1 h) leaves for an induction period of 1 s. The JIP test parameters and the numerical processing of the PF, DF, and MR signals were performed according to (Strasser et al. 2010; Kalaji et al. 2012).

Simultaneous registration of both PF and DF is based on consecutive irradiation and darkening of the studied plant leaves to harvest both kinds of signals – PF and DF, respectively. M-PEA measuring protocol includes dark periods not exceeding 25% from registration time.

Results and Discussion

Drought stress effects on electron transport in drought sensitive bean (Phaseolus vulgaris L.) leaves

In our work we estimated the effect of drought by the changes in PF, DF and MR (Figure 1). On Figure 1A induction curves of PF registered in bean leaves were presented. Up to 10-day drought, significant changes were not found in F_{max} and F_0, while 12 day treated plants display visible decrease in F_{max}.

The properties of the microsecond DF induction curve are described in Strasser et al. (2010). This microsecond DF was mainly determined by activity of oxygen evolving complex of PSII (Goltsev et al., 2009). DF induction curves of microsecond DF component are presented on Figure 1B. A maximum about 7ms was registered (denoted as I_1, according to nomenclature introduced by Goltsev and Yordanov (1997). Further a drop and following formation of the shoulder between 100 – 200 ms (maximum at I_2) and decrease in

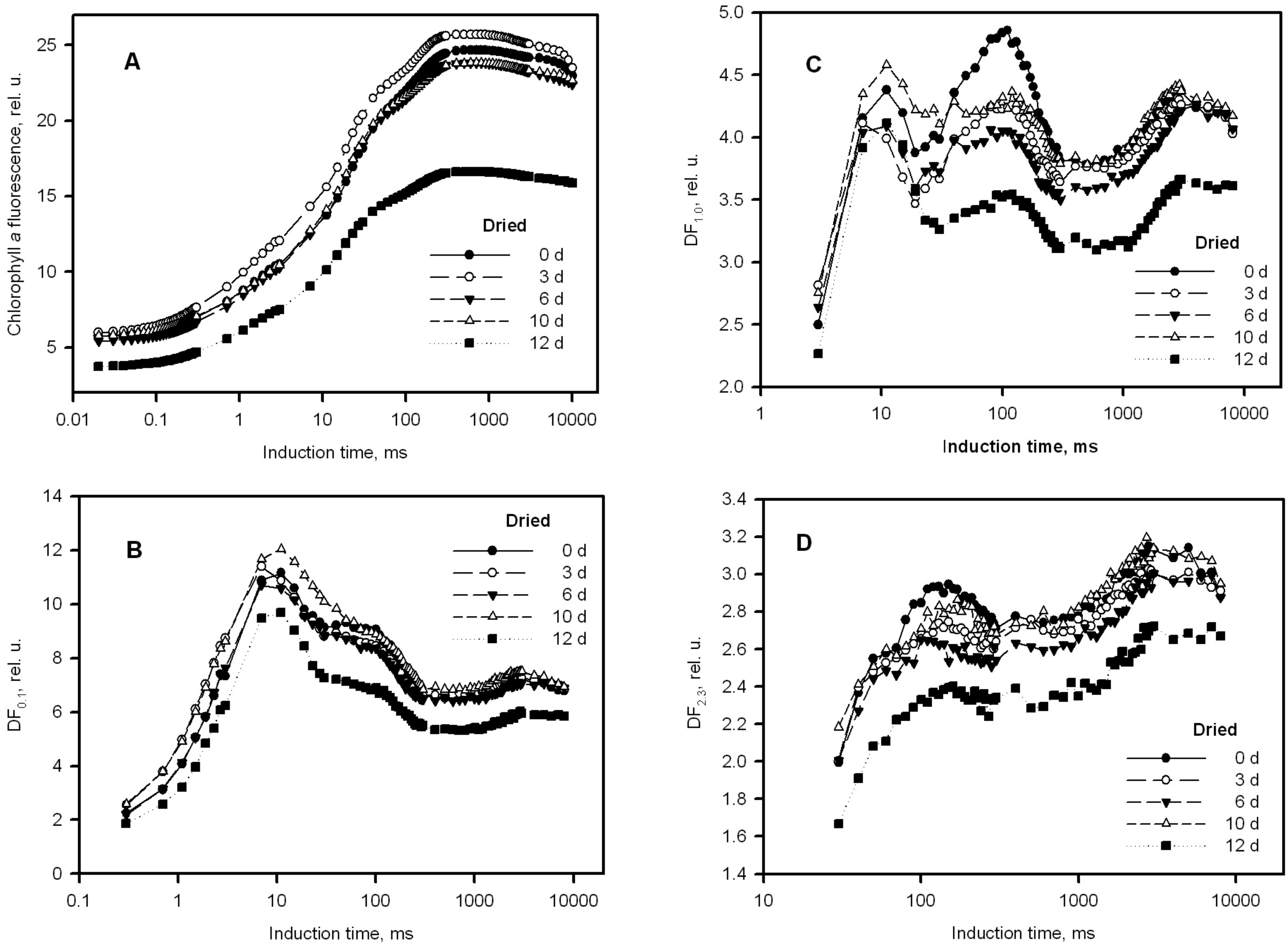

Fig. 1. Drought effect on induction kinetics of PF (A) and DF (B-D) in leaf discs from drought sensitive bean plants. The measurements were carried out by M-PEA fluorometer (Hansatech Instruments, Ltd, UK) at exciting light of 2000 µmol hv.m^{-2}.s^{-1}. The plants were dark adapted for 1 h before measurement. DF signals were recorded during dark intervals of 0.02-0.09 ms (B), 0.1-0.9 ms (C) and from 1.0 ms to 2.3 ms (D). Irrigated (0 d) and desiccated for different periods (3-12 d) plants were analyzed

DF to minimum (D_2) about 300–500 ms was observed. The rise to I_1 reflects the formation of light emitting states in PSII reaction centers ($ZP680^+Q_A^-$), while the next drop to D_2 reveals partial closure of PSII reaction centers (see Goltsev et al. 2009). Intermediate delaying in this drop about 100–200 ms is related with an activation in intersystem chain electron transport reactions in the case of prevailing oxidized state of primary PS1 electron donor, $P700^+$. Similarly, to changes in PF the microsecond component $DF_{0.1}$ did not change significantly after drought stress (Figure 1B).

On the other hand, sub-millisecond component of DF ($DF_{1.0}$) reflects the activity of electron transport chain components in PSII acceptor side (Figure 1C). $DF_{1.0}$ component is emitted by PSII reaction centers in $Z^+Q_A^-$ state. Electron transfer beyond c Q_A^- to second semiquinone (Q_B^-) determines related kinetics of $DF_{1.0}$. Sub-millisecond component of DF transients was very sensitive to drought treatment in opposite to microsecond one. For instance, earlier drought period caused a decrease in I_2 maximum. It could be related to enhancement in effective antenna size of PSII on one hand and with inactivation in intersystem electron transport chain on the other.

Similar effect was observed for 200 ms maximum of millisecond DF, $DF_{2.3}$ (Figure 1D). It was supposed that decay kinetics of $DF_{2.3}$ is related with electron transfer from Q_B^{--} to PQ-pool and further opening of the closed PSII reaction cen-

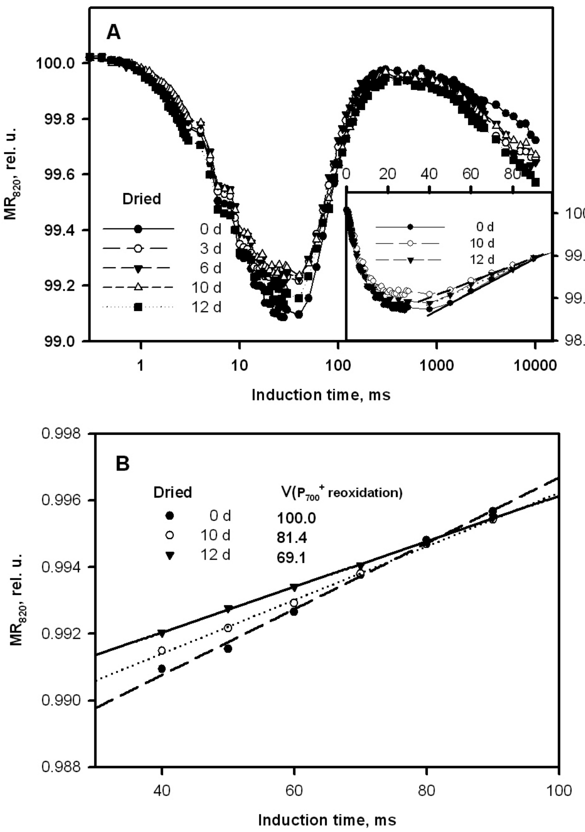

Fig. 2. Drought effects on light induced changes in MR_{820} in bean leaves (A). Inset in figure reveals changes during the initial 100 ms period of illumination in linear scale. Fitted data from linear part of signal increase in interval 20 to 100 ms is presented in panel B

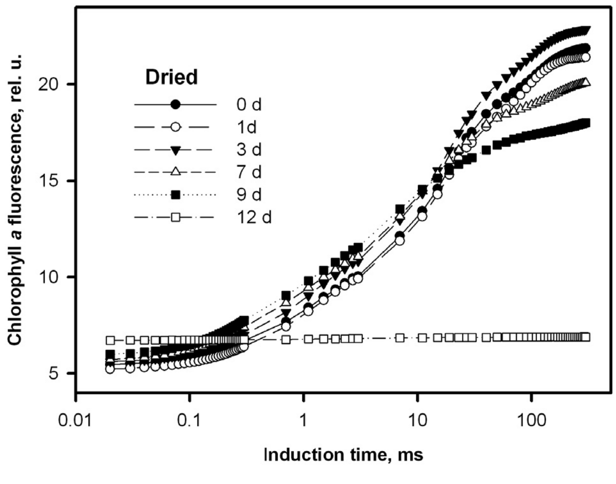

Fig. 3. Drought effect (0d to 12d desiccated plants) on prompt fluorescence transient in plantain leaves. Measuring conditions are as in Fig. 1

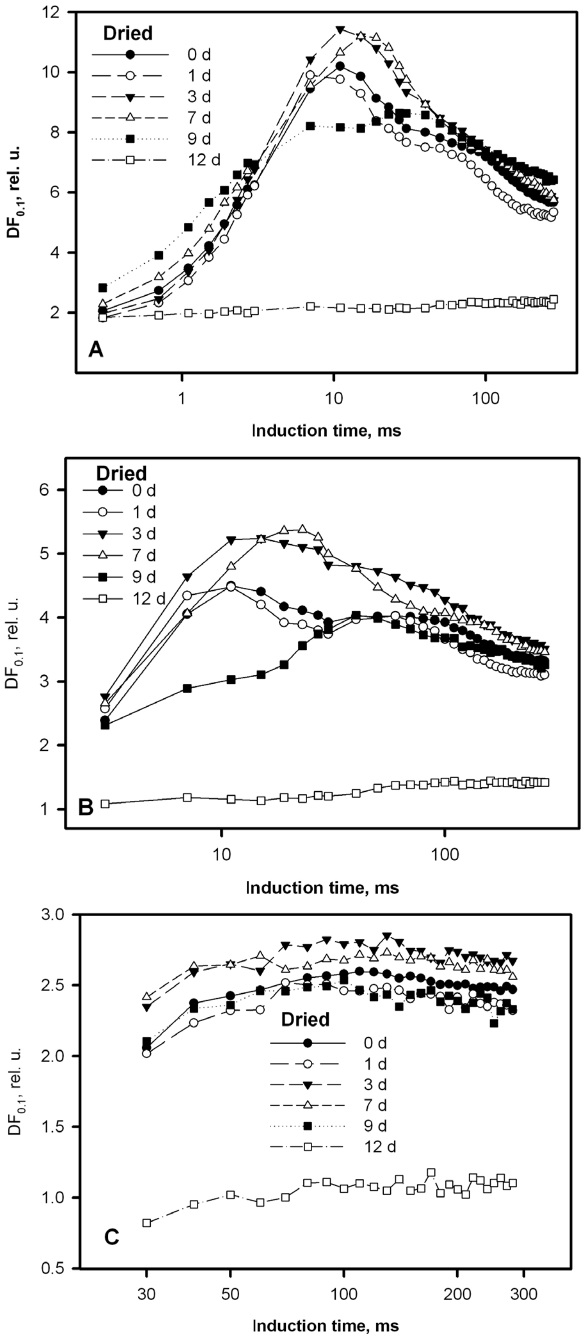

Fig. 4. Drought effect (0d to 12d desiccated plants) on delayed fluorescence transient in plantain leaves. Exciting light during registration of PF induction was 2000 µmol hν.m^{-2}.s^{-1}. Leaves were dark adapted for 1 h before measurement. DF measurements were registered during dark intervals of 0.02–0.09 ms (B), 0.1–0.9 ms (C) и от 1.0ms до 2.3 ms (D). Irrigated (0 d) and desiccated for different periods (3–12 d) plants were presented

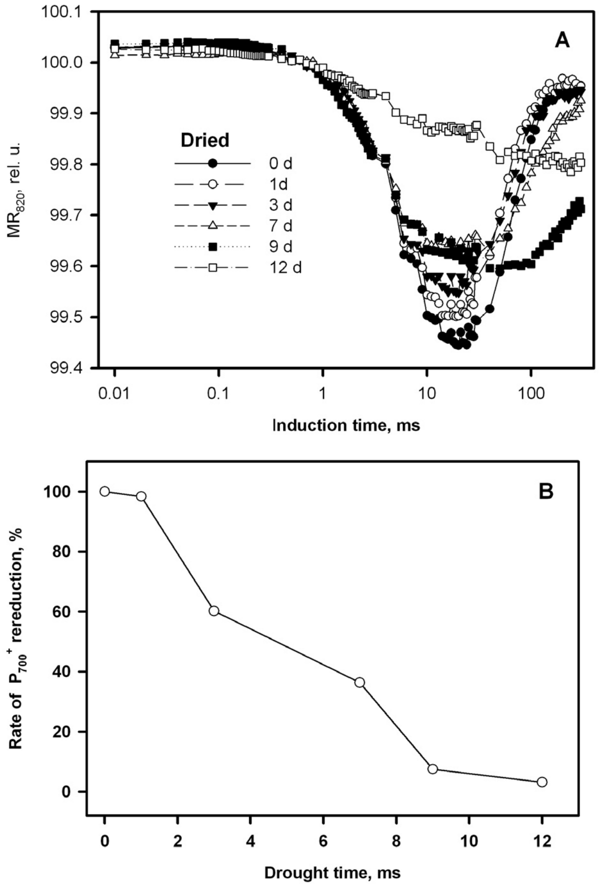

Fig. 5. Drought effects on light induced changes in MR_{820} in plantain leaves (A). (B) Rate of $P700^{+}$ reduction as depended by drought treatment

ters in state $Z^{+}P680Q_A^{-}Q_B^{=}$. Slower phases prevailed in millisecond DF induction kinetics. $DF_{2.3}$ component is related to dynamics of exchange of PQ molecules in Q_B pocket in PSII acceptor side and following oxidation of PQ pool. The rate of exchange is closely related with viscosity of thylakoid membrane, which on the other hand depends on water content around membrane. In addition, the formation of $DF_{2.3}$ was determined by recombination between electrons in reduced Q_A^{-} and oxidized states in PSII donor side.

By estimation of MR_{820} kinetics (Figure 2) we can observe the dynamics of electron transport reactions beyond PQ-pool. Electron transfer in both donor and acceptor side of PSI did not depend on drought effect. Main drought induced changes are related with redox state of primary donor in PSI reaction center, P700. A slower rate of electrons was established via electron transfer through PQ carriers. Linear regression of the fastest part of MR_{820} increase represented on Figure 2 shows delayed reduction of $P700^{+}$ after 30% drought stress.

Drought effect on electron transport in drought tolerant plantain (*Plantago major* L.) *leaves*

Plantain (*Plantago major*) is plant with enhanced drought tolerance. Plantain revealed higher rate of photosynthesis at extended interval of water potentials and temperatures (Mudrik et al., 2003). This plant is characterized by high water use efficiency.

12 days drought treatment caused different dynamics of changes in PF and DF and in MR_{820} as well (Figures 3 – 5).

In opposite to drought sensitive bean plants subjected to 12 day drought treatment, photosynthetic apparatus of plantain reveals earlier response to drought. It was observed visible decrease in maximal fluorescence, F_m (Figure 3). It is possibly a result of non-photochemical fluorescence quenching.

The most sensitive parameter for estimation of drought stress effects could be considered second maximum of submillisecond DF, which appeared at slower induction times (Figure 4B), while $DF_{0.1}$ and $DF_{2.3}$ the components did not change significantly. Such reduced appearance of second maximum could be explained as decreased rate of intersystem electron flow in tolerant *Plantago* plants. Such suggestion is well supported by changes in MR_{820} signal (Figure 5B). Because of delayed intersystem electron transport, the rate of $P700^{+}$ reduction was significantly decreased.

Conclusion

The results show that drought induces slower electron transport rate in intersystem chain in drought tolerant plant *Plantago major* as compared to the sensitive plant species *Phaseolus vulgaris.* It could be considered as a part of protection mechanism against drought on the level of photosynthetic electron transport. The similar sensitive stress reaction was demonstrated in desiccation tolerant plants *Haberlea rhodopensis* (Strasser et al., 2010). Recently, many evidences were provided concerning the protective role of PSI cyclic electron transport from damages induced in photosynthetic membranes by drought stress (Bukhov and Carpentier, 2004). These results allow us to propose that reduced intersystem electron flow represents the concerted adjustment between the linear electron transport chain and cyclic electron flow around PSI.

Acknowledgements

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