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INTERACTION BETWEEN STRESSES

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> *"If we wish to understand life we must study death"*
> Levitt, J., 1980

Summary. Normally, under natural conditions, plants are subjected to the influence of at least two different stress factors. The physiological responses of plants, exposed to two subsequent stress factors differing in their intensity or duration are reviewed. In the experiments presented here, the effects of some natural (water depletion, extreme temperatures), and anthropogenic (UV-B irradiation and herbicides) stresses, applied alone and in combination were studied. As a measure of the interaction between stresses, the changes in biometric parameters, the levels of some oxidative stress markers and activity of defence enzymes were monitored in pea, wheat, or maize seedlings (grown as water culture) and in Arabidopsis plants. The relationships between the metabolic changes observed, and the degree of cross-synergism or cross-adaptation to the interacting stresses are discussed.

Key words: Stress interaction, Active oxygen species, Stress markers, Cross-synergism, Cross-adaptation

Abbreviations: MDA – malondialdehyde

Abiotic and biotic stresses cause alterations in the normal physiological processes of all plant organisms, including the economically important crops. Plant damage and decrease in their productivity take place most often due to naturally occurring unfavourable factors of the environment (natural stress factors) - extreme temperatures; water deficit or abundance; increased soil salinity; high solar irradiance; early autumn or late spring ground frosts; pathogens etc. Recently, along to these factors plant organisms are imposed to a large scale of new stressors related to human activity (anthropogenic stress factors) – toxic pollutants such as pesticides, noxious gasses (SO_2, NO, NO_2, NO_x, O_3 and photochemical smog); photooxidants; soil acidification and mineral deficit due to acid rains; overdoses of fertilizers; heavy metals; intensified UV-B irradiation etc. (Fig. 1). All these stresses decrease the biosynthetic capacity of plant organisms, alter their normal functions and cause damages which may lead to plant death (Lichtenthaler 1996, Levitt 1980).

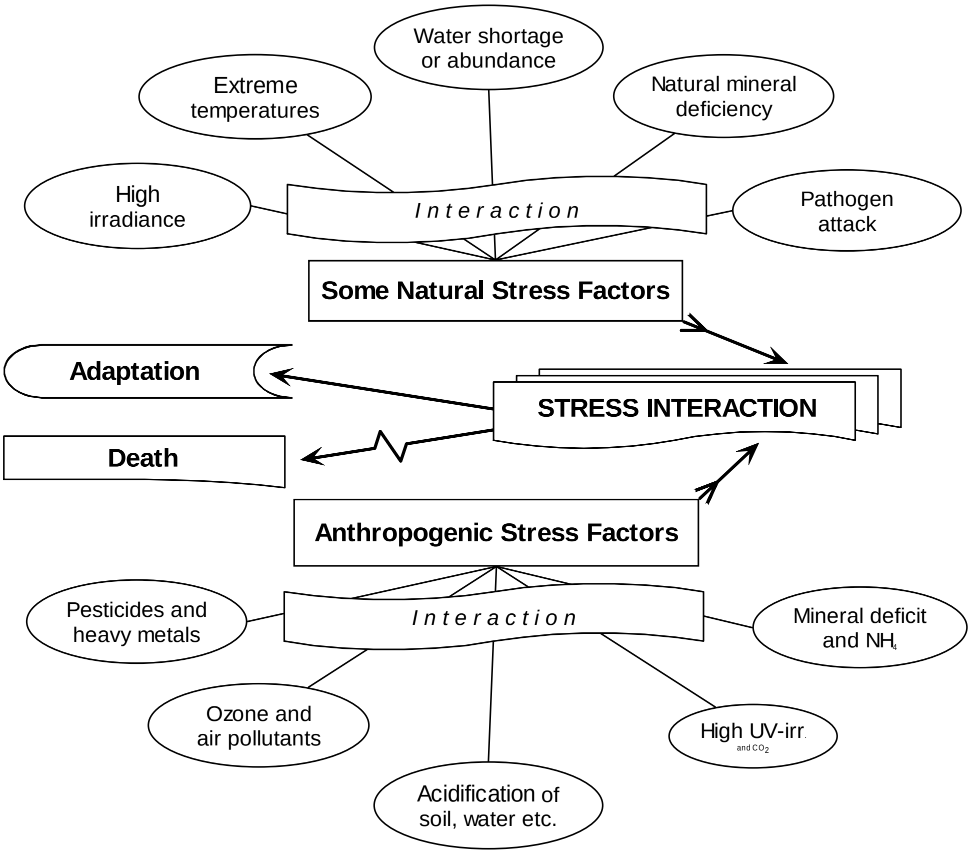

Fig. 1. Stress factors and interaction between them.

Independently of the type of stress influence (natural or anthropogenic) the accumulation of reactive oxygen species is an undeniably established fact. It is well known that water deficit (Sell et al. 1992), low temperatures (Badiani et al., 1993; Bridger et al., 1994; Wise and Naylor, 1987), application of pesticides (Babbs et al., 1989; Banas et al., 1993; Kenyon and Duke, 1985; Sergiev et al., 2000; Ivanov, 2003; Ivanov et al. 2003a, b), noxious gasses (Wingsle and Hallgren, 1993), radiation (Dunning et al., 1994), heavy metals (Pahlsson 1989), acid rains (Velikova et al. 1998, 2000), high solar irradiation (Lichtenthaler 1988; 1996) etc., cause an increased production of toxic oxygen species (O_2^{-}, H_2O_2, $^{\bullet}OH$, $^{1}O_2$), which are highly detrimental

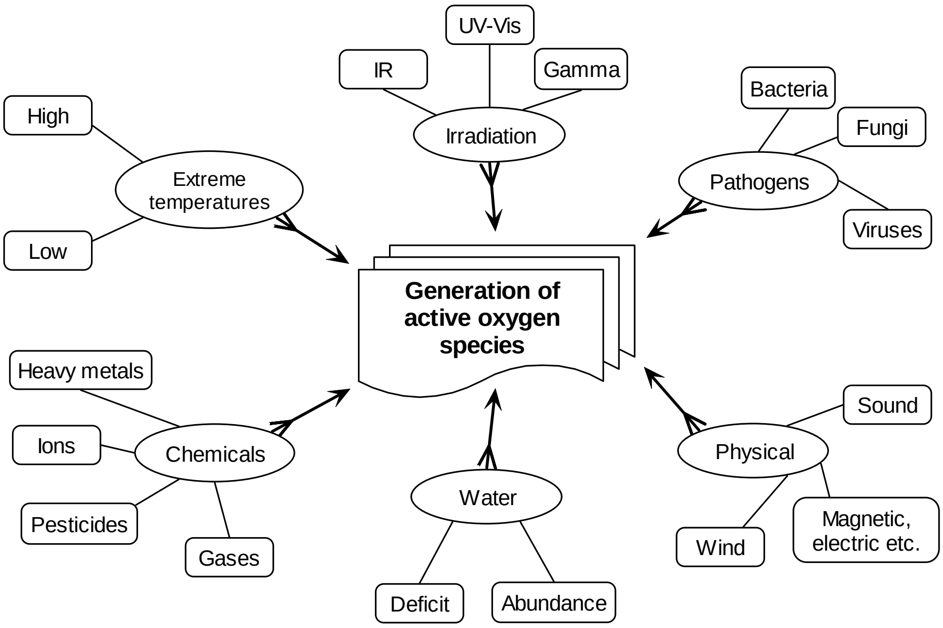

Fig. 2. Agents leading to generation of active oxygen species.

to all biological systems (Fig. 2), i.e. an oxidative stress occurs (Foyer et al. 1997; Halliwel and Gutteridge 2002).

However during their phylogenesis plant organisms have developed a complex of antioxidant protective systems in order to cope with all these destructive effects (Fig. 3). In general the plant antioxidative systems can be divided as follows:

- Lipid-soluble, membrane associated antioxidants – α-tocopherol, β-carotene, which directly quench free radicals of lipid peroxidation (triplete chlorophyll and $^1O_2$);
- Water-soluble antioxidants – glutathione and ascorbate, taking part in the detoxification of O_2^- and H_2O_2;
- Enzymatic antioxidants – superoxide dismutase (EC 1.15.1.1), catalase (EC 1.11.1.6), peroxidase (EC 1.11.1.7) and enzymes belonging to the ascorbate-glutathione cycle.

By activation of some or all of these systems the plant organisms are capable of overcoming oxidative stress. However, in the case of prolonged or acute, even short stress the capacity of the defense systems becomes exhausted or overloaded and this leads to considerable damages and even to plant death.

The importance, actuality and inevitability of the unfavorable environmental natural and anthropogenic factors, as well as their negative impacts on physiological processes and plant productivity have drawn the attention of a number of investigators

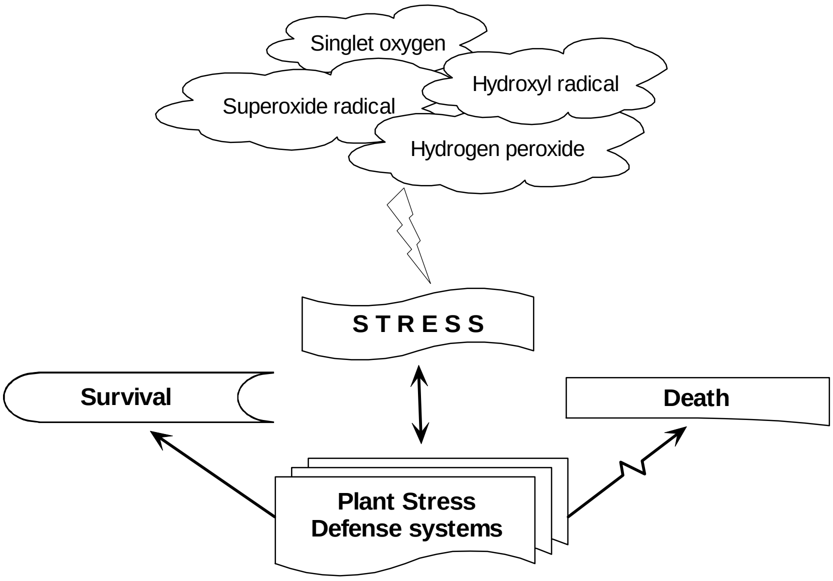

Fig. 3. Active oxygen species and their general impact on the plant defense systems.

to study the problems of plant stress. The research in this area is routed mainly into five directions:

- Studies on the physiological response of the plant organism subjected to a single stress factor;
- Comparison of the effects of a single stress on several plant organisms, differing in their species, variety or genetically determined resistance to the respective stress factor;
- Searching for possibilities to decrease the unfavorable consequences caused by a particular stress factor by induction of adaptation or by means of application of xenobiotics;
- Selection using cell cultures and conventional plant breeding;
- Genetic engineering for improved stress tolerance.

In most of the large number of papers published in this area the effect of a single stress factor (low or high temperature, salinity, radiation, herbicides, water or mineral deficit, heavy metals, acid rain etc.) on the physiological status of the plant organism has been traced, and/or the possibility of alleviating the stress effects is being investigated (Levitt 1980; Georgiev et al. 1991; Zheleva et al., 1994; Yordanov et al., 1986, Yordanov 1992, Velikova et al. 1998; Todorov et al., 1998; Georgiev and Iliev 2000; Velikova et al., 2000; Fedina and Benderliev 2000; Ananieva et al. 2002; Hristova and Popova 2002, etc.).

However, under natural conditions plant organisms are hardly ever subjected to a single stress factor, rather, the impact of a particular stress is accompanied by additional unfavourable issues. For instance, the high solar irradiation, which causes a decrease in the photosynthetic rate by photoinhibition and photooxidation of the plastid pigments is usually accompanied by a heat stress, water deficit and desiccation. The negative effects of the high solar irradiation are additionally favoured by the presence in the air of toxic wastes such as NO, NO_2 and some low molecular weight organic compounds, which by photochemical reaction are transformed into highly reactive oxidative agents such as ozone and peroxyacylnitrates. Most often the simultaneous action of several stress factors elevates their deleterious effect so it considerably exceeds the simple additive effect of their action alone (cross-synergism). For example, in the case of combined application of SO_2 and NO_x the toxic effect is much stronger than the additive effects of both gasses applied alone mainly because SO_2 inhibits nitrate reductase which is responsible for the detoxification of NO_x (Whitmore and Freer-Smith 1982). Similar synergism in the damage levels has been observed in maize treated with atrazine, grown under elevated soil humidity and low temperatures (Stefanovic and Zaric 1991), and in *Brassica napus* seedlings cultivated under water stress and phosphorus deficit (Flasinski et al. 1986). The combined influence of anthropogenic (air pollution) and natural (prolonged drought along with high solar irradiation) stress factors are the main reason for the death of a number of woodland species in Central Europe for the period 1983–1987 (after Lichtenthaler 1988).

On the other hand, there are cases when the plant organism subjected to a single stress agent is capable to increase its resistance to subsequent unfavourable impacts (cross-adaptation). A common example in this sense is the complex relationship between the water deficit and the increased resistance to low temperatures described in citrus (Yelenowski 1979a, b), rye (Siminovitch and Cloutier 1982), wheat (Cloutier and Siminovitch 1982; Willemot and Pelletier 1979), cornel-tree (Chen et al. 1977) etc. Water deficit diminishes the sensitivity of bean plants subjected to ozone treatment (Bender et al. 1991; Langebartels et al. 1991). The short water deficit induces a dehydration and loss of turgor which by itself decreases ice-formation after a subsequent low temperature stress (Palta 1990). Yordanov et al. (1986, 1987) established that the short treatment of bean plants with high temperature (45ºC) stimulates their capacity to survive at 55ºC – a temperature at which the control plants die. Nevertheless the fact that increased UV irradiation alone renders negative effects on the growth and productivity of number of crops – wheat, rice, soybean (Tevini and Teramura 1989), and in combination with some other stress agents its deleterious action is even stronger (Teramura 1986), Dunning et al. (1994) established that the treatment of *Rhododendron* with UV-B increases its resistance to low temperatures probably because of the biosynthesis of phenolic compounds induced by UV-B which by themselves are acting as free-radical quenchers.

The first investigation on the interrelations between stresses have been announced by the pioneer in plant stress physiology – Jacob Levitt in the 30-years of the last century. Presently, a number of research groups from all over world are investigating this problem. Independent of their efforts, however, there are still large “white spots” in the picture presenting the nature as well as the physiological consequences of the interaction between two and/or more stress factors.

While the investigations on a single stress agent present detailed characteristics of the physiological status of the plant organism and those induced by the respective stress factor metabolic changes, almost nothing is known about the mechanisms which lead to the development of cross-adaptation or cross-synergism. The published data in this sense are limited and relate mainly to a description of the observed physiological effects and the proposed explanations are often ambiguous. This of course is not surprising at least for several reasons:

- A single stress agent causes changes in several parameters. Depending on the intensity and/or the duration of the impact, the effects diverse from those stimulating through inhibition and to plant death. On the other hand, stressors with different origins (natural and anthropogenic; biotic and abiotic) influence in a similar way a single parameter – for example plant growth, photosynthetic processes, synthesis of stress-related proteins, activities of some defense enzymes, levels of stress markers etc.
- The image becomes more obscure when interaction among the stress factors takes place. The stressors can act together or subsequently – with different durations and intensities. Moreover, the diversity of the nature of stress agents and the possible combinations among them additionally makes the problem more difficult to be solved.
- There is still a “lack of a well defined model system” – most of the investigations on cross-adaptation or cross-synergism are done with plant explants (Dunning et al., 1994; Pomeroy and Siminovich 1971; Siminovich and Cloutier 1982; Teramura 1986) and the conclusions are not always applicable to intact plants.
- A discrepancy between the data obtained in laboratory and field conditions exists, probably because of the uncontrolled conditions of the experiments under filed conditions – for example, in the case of established induced-resistance to low temperatures after a mild water deficit (see above), the experiments of Koo (1981) and Layne and Tan (1984) demonstrate that under field conditions citrus and peach trees grown in irrigated areas survive the winter months better than plants subjected to a water shortage.

A number of questions still remain to be answered – to what extent the observed physiological response is specific; which regulatory mechanisms within the plant organism are involved; which metabolic processes are affected and what metabolic changes occur after the subsequent or simultaneous application of two or more stress agents; how the established cross-interaction (adaptation or synergism) could be applied in the agricultural practice. Additionally, investigations in this area could also provide additional information on the specificity of action of the single stress factors.

The aim of the current review is to summarize some of the data obtained by our group on the problem of interaction between environmental stresses.

The model systems used are presented on Fig. 4. The investigations were carried out on different plant species (pea, wheat, maize, *Arabidopsis thaliana*) and stress fac-

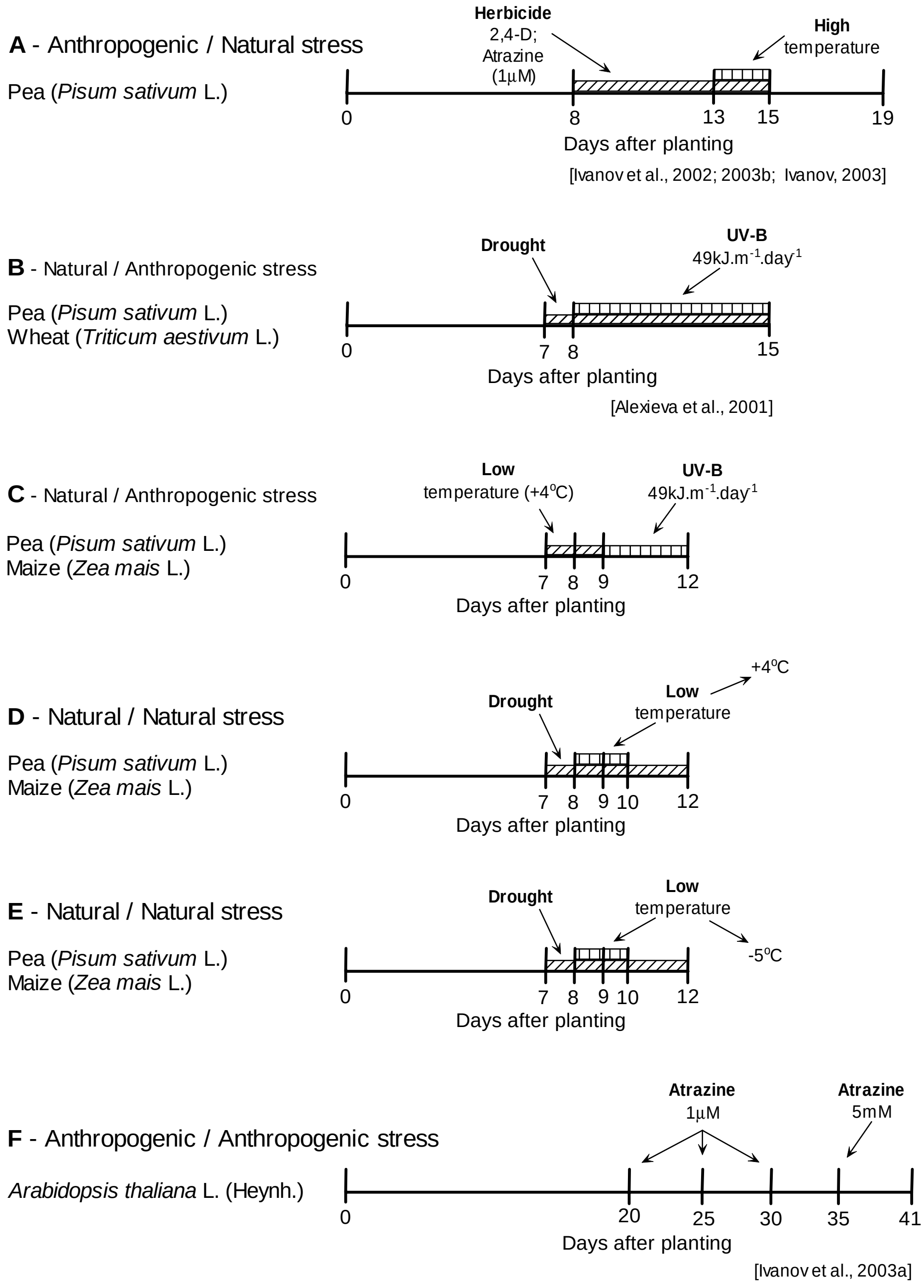

Fig. 4. Model systems and stress factors investigated.

tors with different intensity, duration and origin (natural and anthropogenic) were applied in various combinations (Alexieva et al. 2001; Ivanov et al. 2002; Ivanov 2003; Ivanov et al. 2003a,b).

To characterize the plant physiological status and the effects of the treatments the following parameters were measured – free proline content (Bates 1973), level of lipid peroxidation (Dhindsa et al. 1981), electrolyte leakage (by Dexter et al. 1932, with modifications) and endogenous hydrogen peroxide content (Alexieva et al. 2001) – which are accepted by many authors as markers of stress impact. In general, it is considered that the increase of their amounts is an indication of unfavorable alterations in the plant organism. With exception of free proline (Kuznetsov and Shevyakova 1999), the rest of the stress markers develop as a result of oxidative processes in the plant (Halliwell and Gutteridge 2002).

In all investigations the dynamics of changes of the parameters were followed during the stress program and the recovery period. In the current paper only data of the last measurement are presented.

One of the most representative markers for membrane destruction after free-radical chain reactions is the formation and accumulation of malondialdehyde (MDA), an end-product of peroxidation of the unsaturated membrane fatty acids (Halliwell and Gutteridge 2002). In all model systems investigated the single and combined application of both stress factors leads to increased amounts of MDA (Fig. 5). On the other hand, considerable differences between the model systems can be seen – for example, after the treatment of *Arabidopsis* with low and high concentration of atrazine (Fig. 5F), application of subherbicide concentrations of atrazine and 2,4-D and high temperature on pea (Fig. 5A) and drought and low negative (–5 ºC) temperature on pea and maize (Fig. 5E), the effects of the single application of the particular stressors is lower than those caused by their combined application (presented as a percent of the respective control). In the contrary, after the preliminary mild drought (10 % PEG in the growth medium) and subsequent UV-B irradiation (pea and wheat; Fig. 5B), or additional treatment with low positive temperature (+4 ºC) (pea and maize; Fig. 5D), the levels of MDA in the combined treatments is lower than those determined after the application of the stress factors alone. Similar tendencies were observed also in the combination of low positive temperature and UV-B (pea and maize; Fig. 5C).

As already mentioned, the increase in the amounts of the investigated parameters is an indication of a deleterious stress effect. In order to be able to compare all parameters measured, the data are presented as a “percent of cumulative effect” - expressed as a correlation between the effect induced by the combined application of both stressors (% to the respective control) and the effect of the stress agent provoking more considerable changes in the parameter investigated. For instance, in a case of two stressors A and B and if changes induced by them are A=120%, B=140% respectively, and after their combined application the effect is A+B=160%, then the % of cumulative effect is $(A + B)/B \times 100 = 114.3$. By using of this expression, when the % of

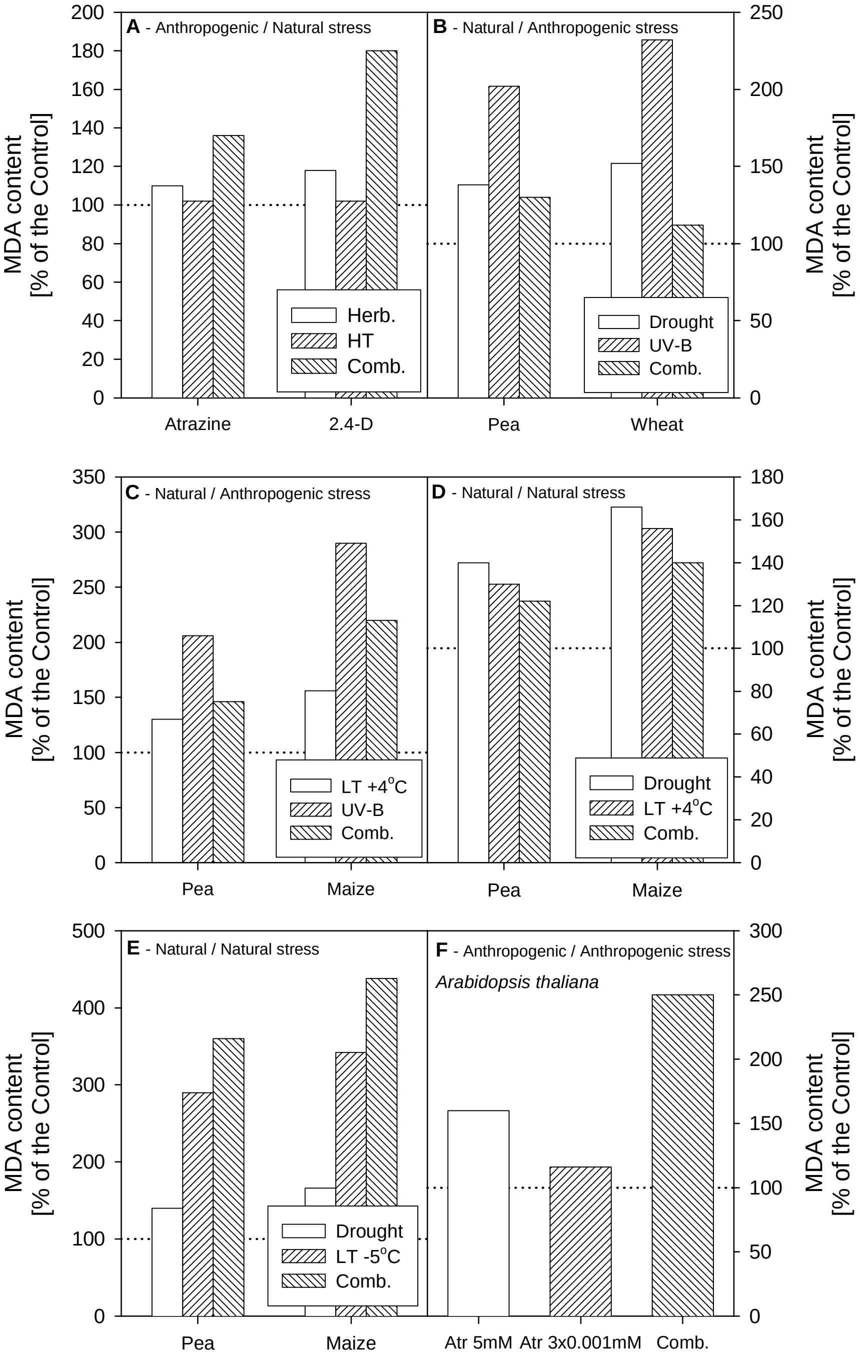

Fig. 5. Effects of stress factors and their combinations on the levels of lipid peroxidation (as MDA equivalents).

cumulative effect is >100 we assume this as a cross-synergism, and in the opposite case, if it is <100, then there is a cross-adaptation.

The data presented in a Fig. 6 (% of cumulative effect) show that the changes observed in the levels of the stress markers give a reliable indication about the results of the interactions between the stresses investigated in the different model systems. The increased percentage of cumulative effects named by us cross-synergism is observed in the following model systems: pea plants treated with low concentrations of atrazine, 2,4-D and high temperature (Fig. 6A; Fig. 4A); pea and maize plants subjected to drought and freezing stress (Fig. 6E; Fig. 4E); *Arabodopsis* treated with low and high concentrations of atrazine (Fig. 6F; Fig. 4F). A cross-adaptation was observed in pea and wheat plants subjected to drought and UV-B (Fig. 6B; Fig. 4B), and pea and maize - drought and low positive temperature (Fig. 6D; Fig. 4D). The observed changes in the amounts of the stress markers investigated with small exceptions are unidirectorial. Nevertheless the definite tendencies observed in the above mentioned combinations of stress factors, show that in some species specific physiological responses occur. For example, after the subsequent treatment of pea and maize with low positive temperature and UV-B, in pea plants a tendency of cross-adaptation was observed (Fig. 6C). On the other hand, the maize plants appear to be more sensitive to the applied stress agents and the changes in the stress parameters tend to a cross-synergism (Fig. 6C).

The current data are supported by the biometrical parameters measured (fresh and dry weight) and the content of plastid pigments (data not shown), as well as the fact that there were distinct visual differences between treatments.

According to us, the generalized data presented here show that any of the investigated anthropogenic factors – herbicide, UV-B (independently of the nature of the additional stressor, applied in advance, together with, or subsequently) lead to considerable, often irreversible damages to plant organism, this is most probably due to the fact that during their phylogenesis plant organisms have “elaborated” endogenous defense systems against the naturally occurring unfavorable factors of the environment, but the intensive human activity (i.e. the anthropogenic stresses) could lead to unpredictable sequels.

Summarizing the published on the problem of interaction between stresses, as well as our investigations, it can be concluded that a short and mild treatment with a single stress agent may negate the deleterious effects of a subsequent stress – most probably because the first one induces some of the defense mechanisms thus leading to an increased resistance to following unfavorable factors (cross-adaptation). On the other hand, a similar treatment with a single stressor could also lead to an enhanced susceptibility of the plant organism to the same or different stress factor, which could lead to irreversible damages (cross-synergism).

The excellent concept presented by Lichtenthaler (1996), based on the stress theory of Selye (1936) represents four general phases of the stress reaction, each of

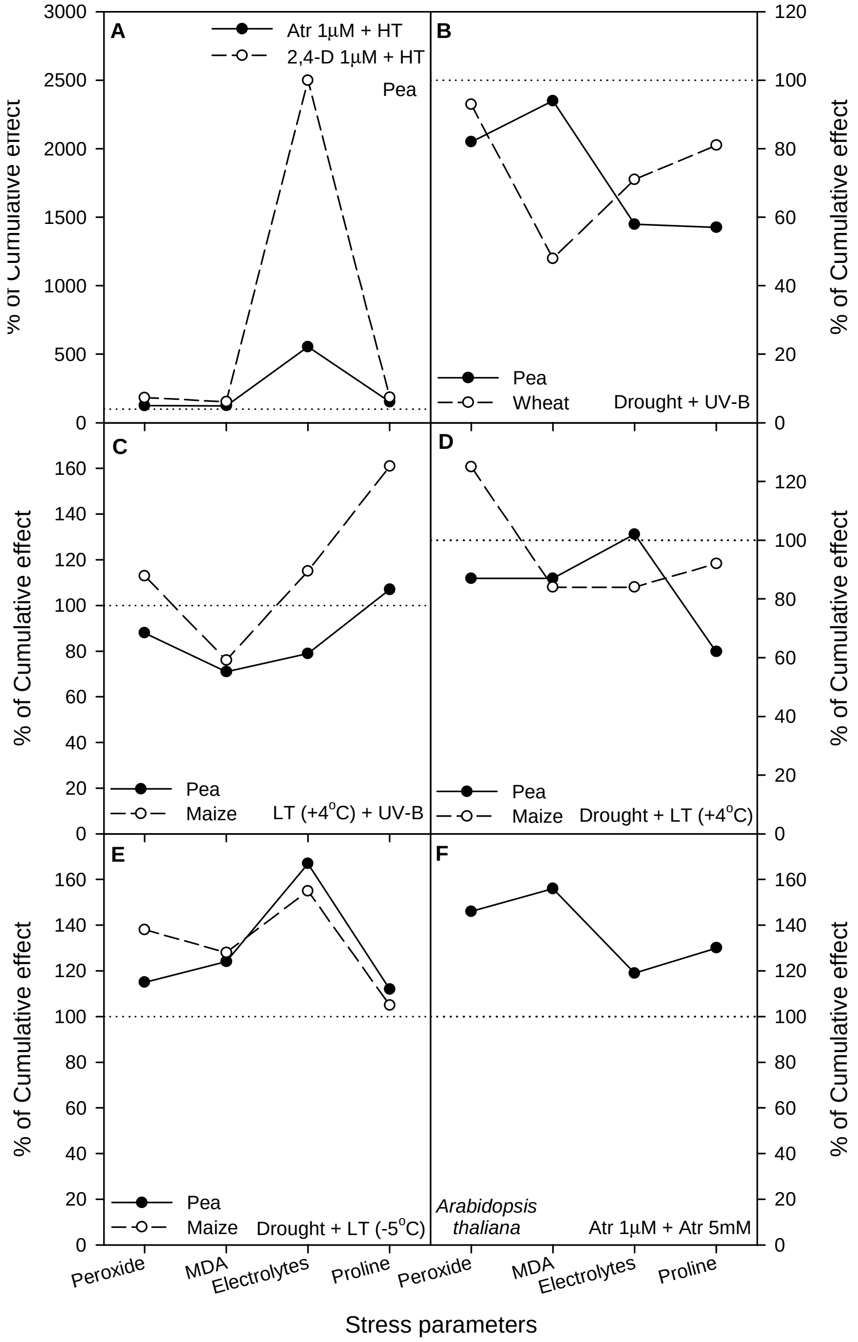

Fig. 6. Cross-synergism and cross-adaptation expressed as % cumulative effects on hydrogen peroxide, lipid peroxidation, ion fluxes and free proline content after combined application of different stress factors (see the text for details).

them characterized by different physiological responses of the plant organism.

1. The so called signal reactions take place – deviations from the normal function, reduced vitality, the catabolic processes are more intensive than biosynthesis;
2. The adaptive and recovery processes are activated;
3. Exhaustion phase – the intensity of the stress factor overcomes the capacity of the plant organism to sustain the stress, chronic damage occurs leading to plant death
4. After the cessation of the stress impact the physiological functions of the plant organism recover wholly or partially.

The level of alterations depends mostly on the intensity and the duration of the stress impact, but the result deformations could be reversible (elastic) or irreversible (plastic). The reversible deformations are assumed to be a normal fluctuation in the physiological processes in response to the stress – for example, the inhibited vegetative growth in some plant species due to low positive temperatures is restored after the temperatures are increased. Of course in a case of continuous stress irreversible deformations could occur.

Summarizing the literature as well as our data we propose a complementary scheme to that published by Lichtenthaler (1996), which takes into account the physiological reaction of the plant organism imposed to multiple stress factors (Fig. 7). A subsequent stress factor could additionally affect the physiological status of the already stressed plant organism in any moment of the dynamics of its stress response. Under these conditions, the overall physiological state of the plant could be enhanced thus achieving a cross-adaptation or it could be demolished - a cross-synergism of stresses. However, same results can be expected by the action of a single stress agent only, but the presence of an additional stressor could modify the effects of the initial factor into unexpected directions.

Additionally, there are stress agents, which under particular conditions could activate the cell metabolism and increase to some extent the physiological activity without provoking negative consequences, thus acting in favor of the plant organism. For instance, the application of some xenobiotics, especially plant growth regulators could also be assumed as a stress. In this regard, it is well known that the growth regulators of different types (cytokinins, polyamines, jasmonates, ABA etc.) may increase the plant resistance to various unfavorable environmental factors, i.e. they represent an example of a cross-adaptation. Of course in case of prolonged (i.e. repeated) application and especially over-dosage this could cause irreversible disturbance of the plant metabolism (growth processes, photosynthesis etc.) which is leading to inhibition of the physiological activity, induction of preliminary senescence etc., or even plant death. During their onthogenesis all plant organisms are subjected various types of stress, but their physiological response differs in relation to their stage of development. Generally, under stress conditions there are gradually increasing requirements to the plant organism to adjust itself to the environment and this is initiated by disturbance of normal metabolic processes. In some cases plants are able to restore their normal

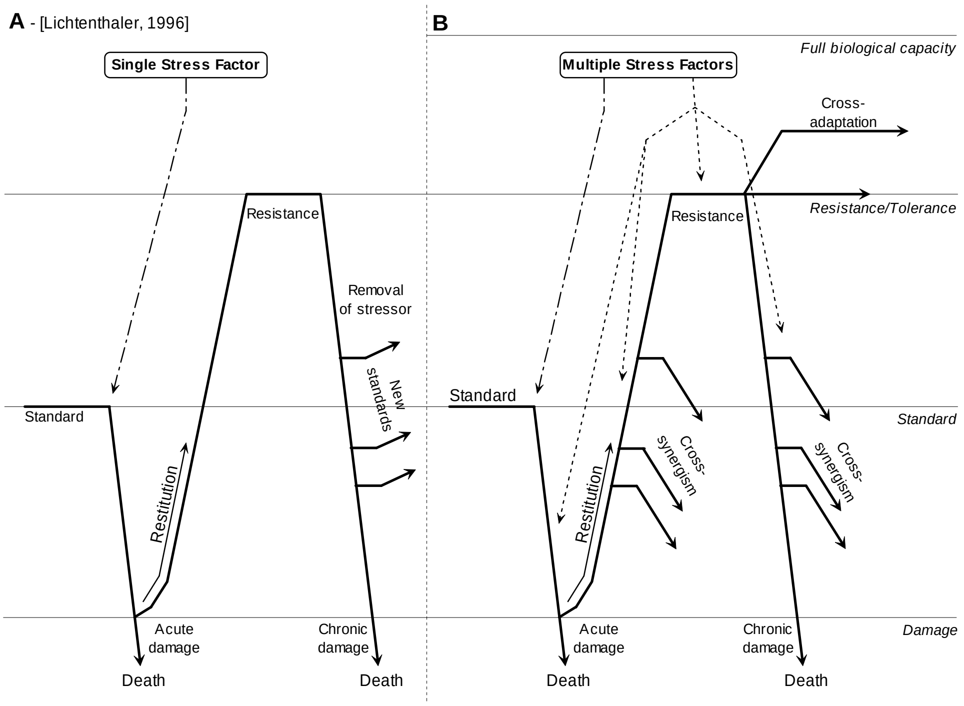

Fig. 7. Plant response to environmental stress. A. Responses of plants to single stress factor (according to Lichtenthaler, 1996). B. Supposed scheme of stress reaction in plants subjected to multiple stressors.

functions, which results in an augmented resistance to the respective stress factor (adaptation). On the other hand, if the stress impact is superior to the adaptive capacity of the plant, then permanent damage occurs. So, the nature of the stress is dual – it is often destructive, but also under some circumstances it could be constructive, and the last appears to be the driving force of the adaptive evolution of the plant organisms (Levitt 1980; Lichtenthaler 1988; 1996).

Future investigation in this area will enlarge the knowledge about the reaction mechanisms of plants imposed to different unfavorable conditions. Additionally, new approaches to the problem of induction of a cross-adaptation or synergism will be revealed and the specificity of the multiple-stress interaction will be better elucidated. Such information will open new possibilities for a deliberate and predictable approach for improvement of the adaptation capacity of plant organisms.

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