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Review

DECAPITATION AND DEFOLIATION AS APPROACHES TO STUDY CONTROL MECHANISMS OF LEAF SENESCENCE AND ITS REVERSIBILITY

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Summary. The removal of the top stem bud independently or in combination with the leaves, known as decapitation, can lead to changes in the physiological state of plants at the same age. The decapitation procedure reverses the symptoms of senescence which makes it an effective physiological approach to study the control mechanisms of delayed leaf senescence and rejuvenation. The process of rejuvenation that develops in the decapitated plants is characterized by increased pigment content and photosynthetic rate. In addition, photosynthetic electron transport, Rubisco activity and photorespiratory metabolism are recovered to the maximal values observed in mature leaves. Thus, through decapitation leaf life span can be prolonged.

Decapitation leads to substantial changes in the biosynthesis, metabolism and transport of plant hormones, especially auxin and cytokinins (CKs). Based on the model of the mutual interactions of auxin and CK in regulating correlative dominance, the process of rejuvenation observed after decapitation could be explained by the increased levels of endogenous CKs due to *de novo* biosynthesis in the nodal stem together with elimination of competition from the epicotyl as a sink for CKs.

Defoliation which is often induced by herbivores, insects, hail, etc., can be applied in studies on the delayed senescence phenomenon. Compensatory photosynthesis and the resulting compensatory growth are the most important processes following defoliation, thus allowing defoliated plants to survive. The altered sink-source relationships in defoliated plants affect photosynthesis, thus producing the overcompensation effect which leads to increased plant fitness. Damaged shoot structure after decapitation/defoliation is compensated by more flexible adjustment to the altered environmental conditions.

Key words: decapitation, defoliation, delayed senescence, overcompensation effect, photosynthesis, rejuvenation.

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Abbreviations: ABA – abscisic acid; BRs – brassinosteroids; CK – cytokinin; Chl a – chlorophyll a; CKX – cytokini oxidase/dehydrogenase; GAs – gibberellins; IAA – indole-3-acetic acid; iP – isopentenyladenine; iPA – isopentenyladenosine; JA – jasmonic acid; IPT – isopentenyl transferase; MeJA – methyl ester of jasmonic acid; NAA – 1-naphthylacetic acid; PCD – programmed cell death; POR – NADPH-protochlorophyllide oxidoreductase; PPFD – photosynthetic photon flux density; PS1 and PS2 – photosystem 1 and 2, respectively; RuBP – ribulose-1,5-bisphosphate; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; SA – salicylic acid; Z – *trans*-zeatin; ZR – *trans*-zeatin 9-riboside; Φ_{PSII} – actual PS2 photochemical activity.

CONTENTS

1. Introduction

Decapitation, i.e. removing the top stem bud independently or in combination with different leaves (Fig. 1) can lead to changes in the physiological status of plants at the same age, which allows studying plant responses at the level of fundamental physiological, biochemical and biophysical processes. Decapitation of plants is a useful experimental approach for investigation of plant senescence and its reversibility (Van Staden and Carmi, 1982; Wilhelmova et al., 2004; Ananieva et al., 2004; Yordanov et al., 2008a, b). In addition, decapitation experiments could serve as a model system in studies of herbivory-induced defoliation (Delaney and Macedo 2001).

Decapitated plants have been widely used as an appropriate model system to study control mechanisms of leaf senescence. The first results of the effect of decapitation on the physiological status

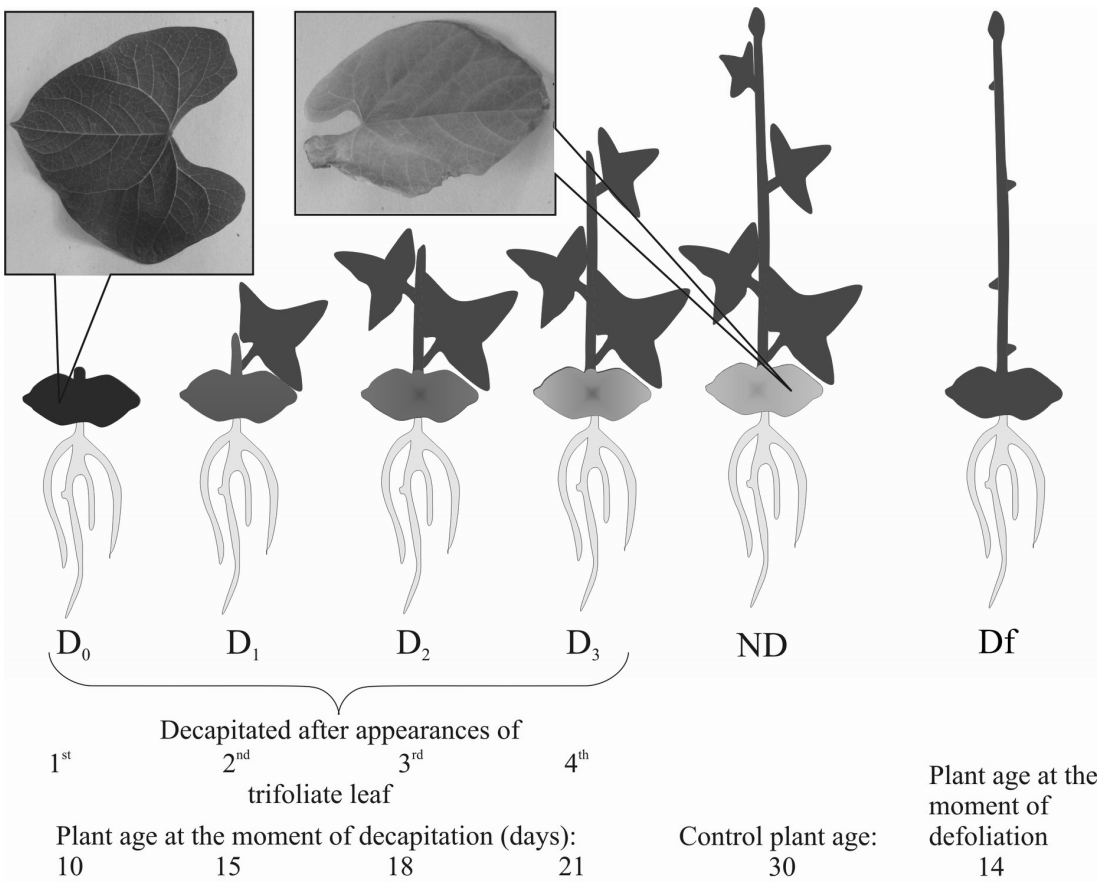

Fig. 1. Experimental scheme. The apical bud was removed after the appearance of the 1st, 2nd, 3rd and 4th trifoliate leaf, respectively, in 10–21-day-old bean plants (variants D_0–D_3) grown hydroponically in a growth chamber at controlled temperature (22–23°C), light intensities (120–130 μmol^{-2} s^{-1} PFD), relative humidity (60–65%) and 12/12h (day/night) photoperiod. All newly formed young trifoliate leaves in the defoliated plants (variant Df) were removed. This figure was published in Journal of Plant Physiology, Yordanov et al. (2008): Preservation of photosynthetic electron transport from senescence-induced inactivation in primary leaves after decapitation and defoliation of bean plants. 165, p. 1956, Copyright Elsevier.

of the plants were received half a century ago by Mothes and Baudisch (1958). They investigated the cytokinin influence on the chlorophyll content and the effects of decapitation on rejuvenation, i.e. regreening and prolonged leaf life span (Mothes and Baudisch, 1958). During the sixties of the last century the physiological changes in decapitated bean plants were extensively studied by Yordanov and collaborators. Our first paper in this field was published in 1967 (Yordanov and Popov, 1967). It was established that decapitation of bean plants above either the cotyledons or the primary leaves considerably delayed their senescence (Yordanov and Popov, 1967; Popov et al., 1969). Moreover, a lot of evidence in literature point to the reversibility of the senescence symptoms (Mothes and Baudisch, 1958; Van Staden and Carmi, 1982; Skadsen and Cherry, 1983; Marek and Stewart, 1992; Wilhelmova et al., 1997; Kutik et al., 1998; Ananieva et al., 2004; Ananieva et al., 2008; Yordanov et al., 2008a, b; Mishev et al., 2009).

Decapitation can also be used to investigate defoliation, which is often induced by herbivores, insects, hail, etc., as well as the delayed senescence phenomena that appear after defoliation (Yordanov et al., 2008a, b). Herbivores cause many injuries to plant organs and affect plant development. It seems that herbivores usually cause negative effects on plant growth and fitness (Crawley, 1989), although in some cases herbivory could benefit plant fitness (Prins and Verkaar, 1992). Decapitation and defoliation positively affect plant performance especially in herbaceous species. They are often accompanied with an increased rate of photosynthesis reflecting the overcompensation effect (Crawly, 1997; Schultze et al., 2005). On the other hand, herbivory can benefit some plants because of plant overcompensatory responses during growth at low or even high herbivory injury levels (Delaney and Macedo 2001). Herbivory does cause some plant damage but not as much as expected based on the injury received. However, due to the overcompensatory plant responses, plant vegetative growth and/or reproductive production increases following herbivory injury, in which plant fitness would be expected to be higher in plants subjected to herbivory relative to plants not subjected to herbivory. The debate about plant overcompensation remains unclear because variations in environmental conditions and genetic variation in plant ability to compensate for injury seem to exist even within a single plant species (Delaney and Macedo 2001). At a broader level, some herbivores may serve as controlling factors of plant population dynamics and distributions (Crawley, 1989 and references therein).

Changes in chlorophyll fluorescence, millisecond-delayed fluores-cence and far-red induced absorption at 830 nm were investigated as a measure of the alterations in PS1 and PS2 electron transport during rejuvenation of decapitated bean plants and senescence of non-decapitated control plants (Fig. 1; Yordanov et al., 2008a, b). An enhanced electron transport rate estimated by Chl *a* fluorescence in the leaves from decapitated plants was observed (Yordanov et al., 2008a). In the defoliated plants with preserved apical buds (variant Df in Fig. 1) the electron transport rate values were similar to those in the decapitated plants with primary leaves only (D_0). The decrease in the

yield of delayed fluorescence as well as the changes in DF thermograms implied acceleration of the electron transport beyond PS2 in the decapitated plants (Yordanov et al., 2008b). In addition, the PS1-driven electron transport as estimated by A830 leaf absorbance changes was also accelerated. Our results showed that decapitation can retard the senescence of primary leaves, expand leaf life span and cause activation of both PS1 and PS2 electron transport rates. It was suggested that modulated sink-source relationships in the decapitated plants could be involved in the changes of the photosynthetic parameters studied (Yordanov et al., 2008a, b).

Changes in the hormonal status that induce rejuvenation were also reported (Wilhelmova et al., 2004; Ananieva et al., 2004), but the intimate mechanisms of the overcompensation photosynthesis in decapitated and defoliated plants remain still obscure. Moreover, responses to defoliation can vary significantly among graminoid, arborescent, boreal and tropical plants (see section 'Defoliation').

This review describes the advantages of the model systems of decapitated or defoliated plants in studies on:

1. Mechanisms of leaf senescence;
2. The delayed senescence phenomenon in leaves;
3. The role of the overcompensation effects after defoliation;
4. Changes in hormonal balance and source-sink relationships causing delayed senescence and the role of apical dominance in the control of senescence mechanisms;
5. Changes in photosynthesis in decapitated plants as a consequence of the overcompensation effect.

2. Senescence, aging and plant growth

Generally, leaf senescence can be simply considered as the final stage of leaf development. Leopold (1980) postulated that the processes of plant senescence led to plant death. Wang and Woolhouse (1982) argued that the threshold point of no return was important in leaf senescence. In contrast, Thomas et al. (2003) defined senescence *per se* as a set of processes that does not ever result in death, but it can result in death through the process generally defined as programmed cell death (PCD). The rapid cell death that accompanies age-related changes in plant metabolism clearly depends on the activation of a genetic senescence program within the plant cells.

Senescence should be distinguished from the process of aging. Aging is a time-dependent, irreversible and deteriorative process leading to death of a whole plant. On the other hand, senescence is an active and reversible process that can be affected by hormones and environmental conditions, and it does not necessarily lead to death (Smart, 1994; Gan and Amasino, 1997). The term "senescence" should be used when describing the development of a plant part (such as leaf).

The leaf senescence process differs between plants with various anatomy (arborescent, graminoid), life cycle (annual, perennial) or habitats (boreal, tropical, geophytes, etc.). In perennial plants, senescence and death of organs such as leaves is often an annual event. Annuals (e.g. *Arabidopsis*), biennials (e.g. wheat) and some perennials (e.g. bamboo) show monocarpic senescence, i.e. fruit set and maturation are directly associated with whole-plant senescence and death. Monocarpic life pattern is characterized

by only a single reproductive event in the life cycle. Monocarpic senescence includes three coordinated processes: (a) senescence of somatic organs and tissues such as leaves, (b) arrest of shoot apical meristems, a form of mitotic senescence or proliferative senescence (see section 6), and (c) permanent suppression of axillary buds to prevent formation of new shoots/branches (Gan, 2007). Other types of senescence (for details see Fischer, 2007) are top senescence (in geophytes, i.e. species with bulbs, tubers, tap roots or rhizomes), deciduous senescence (in some trees and shrubs of temperate climate zones) and progressive senescence (e.g. in evergreen trees). In contrast to annuals, leaf (or whole-shoot) senescence is often not directly associated with seed filling in perennial plants (Feller and Fischer, 1994; Nooden et al., 2004).

Senescence requires active plant metabolism as shown by the activation of specific sets of genes (Gan and Amasino, 1997; Nam, 1997). During leaf senescence, the sum of morphological, physiological and molecular changes is generally referred to as 'the senescence syndrome' which includes visible colour changes, dismantling of chloroplasts, degradation of RNA, proteins and DNA and translocation of macro/micromolecules from senescing leaves to other parts of the plant resulting in the death of the leaf (Bleecker and Patterson, 1997). In contrast, decapitation leads to reversible processes in the leaves (re-greening), thus overcoming the 'senescence syndrome'. Reversibility of leaf senescence has been observed in bean and zucchini plants grown under controlled conditions (Yordanov et al., 2008a, b; Ananieva et al., 2004). The reversibility of leaf senescence as a consequence of the decapitation procedure clearly demonstrates the differences between senescence and aging (whole-plant aging is not reversible) and helps to distinguish between senescence and PCD. The terms 'leaf senescence' and 'PCD' have led to some confusion (van Doorn and Woltering, 2004). Leaf senescence as the visibly observed leaf yellowing and petal wilting has often been accepted as synonymous with the programmed death of the constituent cells. PCD obviously refers to cells which undergo changes following a programme eventually leading to their death. Leaf yellowing can be reversed in some particular conditions. Van Doorn and Woltering (2004) have defined leaf yellowing before the point of no return as "senescence" whereas the process after that point is considered as "PCD", i.e. after passing this point the leaf will die. However, before this point, leaf senescence can be reversed, for instance, by decapitation of the plant. A question arises concerning the natural conditions where clear seasonal changes exist: the alterations of light conditions and temperature activate and drive leaf yellowing especially in temperate regions. Therefore, the controversies referring to the positive or negative impact of defoliation on plant physiological state originate from the changing environmental conditions under which defoliation occurs (section 5. "Defoliation"). Moreover, limited nutrient resources can lead to different responses to defoliation in arborescent and graminoid boreal or tropical plants. It was also established that rejuvenation caused by decapitation was reduced with the progression of the aging process. If decapitation was applied at a later growth stage, it led to a reduced overcompensation

effect of photosynthesis (Yordanov et al., 2008a, b).

Delimitation between senescence and PCD requires searching for effective and reliable experimental approaches. In this respect, modulation of plant life cycle by decapitation allows more detailed studies on control mechanisms of plant senescence as this approach leads to an easy return of the process of senescence. It is well known that decapitation results in rejuvenation. Therefore, it is possible to reverse senescence and thus stop PCD-related processes in the leaves.

3. Decapitation experiments and delayed senescence

3.1. Modification of the leaf senescence program by internal and external factors

Various internal and external stimuli such as age, light, temperature, drought, elevated CO_2, reproduction, plant growth regulators, etc. can modify the leaf senescence program (Jordi et al., 2000). These factors interact with each other in triggering or delaying leaf senescence (Lers, 2007).

In general, phytohormones such as cytokinins, gibberellins and auxins can delay senescence. Some external factors, such as dark treatment, low light acclimation, structure of plant canopy, effect of pathogens, etc. could also delay leaf senescence.

3.2. Low light- and dark-delayed senescence

Whole adult plants placed in darkness for a short time showed delayed senescence (Weaver and Amasino, 2001; Mishev et al., 2009) in contrast to excised leaves in which darkness induced accelerated senescence which could result in plant death (Lu and Zhang, 1998; Oh et al., 2000; 2003). The dark-delayed senescence was regulated by phytochrome and very weak light prevented senescence of leaves (Okada et al., 1992). Dark-induced breakdown of chlorophyll was strongly retarded by continuous illumination with low light intensity, but the effect of light decreased at intensities above 10 μmol m^{-2} s^{-1} PPFD. A brief illumination with red light significantly suppressed degradation of chlorophyll during subsequent dark periods and the effect of red light was nullified by a short irradiation with far red light (Okada et al., 1992). Therefore, degradation of chlorophyll is regulated by phytochrome.

In addition to darkness, the levels of light intensities can also regulate the rate of senescence. There are some controversies concerning the effects of low light irradiation on the rate of senescence. Low light is known to cause senescence enhancement (Lers, 2007). In order to retard senescence, the photosynthetically active light has to be above the photosynthetic compensation point (Veierskov, 1987). On the other hand, Hidema et al. (1991) found that senescence was delayed at low irradiance acclimation of rice plants.

The structure of plant canopy can also modulate the rate of senescence. Saeki (1959) revealed that the formation of the vertical gradient of the rate of light-saturated photosynthesis within a leaf canopy of herbaceous plants was important for leaf adaptation. The vertical gradient of the rate of light-saturated photosynthesis determined a gradient in leaf nitrogen distribution in a plant canopy (Field, 1983). As a consequence, both light and nitrogen gradients can cause different acclimation

in leaf canopy and hence different rate of degradation, i.e. leaf senescence (delayed or accelerated) could depend on the leaf position in the canopy (Hikosaka et al., 1993).

3.3. Biotic factors and delayed senescence

The development of leaf symptoms during plant-pathogen interactions is often accompanied by the appearance of green islands after initiation of the infection which is considered as delayed senescence (Panstruga, 2003). Evidence that host cell death is indeed suppressed during compatible biotrophic interactions can be provided by the "green island" effect: leaf areas around successful infection sites display delayed senescence in comparison with the rest of the leaf tissue. Such heterogeneity in symptoms distribution on the leaf blade induces heterogeneity in metabolic responses to the infection, especially in photosynthesis (Berger et al., 2003).

3.4. Delayed senescence and "stay-green mutants"

At present, the importance of delayed senescence for plant life is not well understood. Moreover, the term "delayed senescence" contains itself some controversy and has not been strictly used in literature. The increasing number of investigations with mutants showing retardation of plant senescence (Oh et al., 1997; Jordi et al., 2000) could help using more precisely the term "delayed senescence". Transgenic plants with delayed senescence have been used to investigate the role of leaf senescence in photoprotection and nutrient remobilization (Jordi et al., 2000). Instead of promoting cell death, senescence-dependent processes may play an important role in preventing premature death. Often leaves are still green when photosynthetic activity starts to decline (Stessman et al., 2002). During this stage, continued capture of light energy by chlorophyll, especially free chlorophyll released from the pigment complexes, could result in premature cell death through the formation of reactive oxygen species (ROS). To prevent photo-oxidative stress, the photosynthetic apparatus has to be dismantled in an ordered manner. It was found that oxygenation of pheophorbide *a* was a key step in chlorophyll breakdown (Tanaka et al., 2003). It was proposed that the inhibition of its activity arrested the chlorophyll breakdown and led to the "stay-green" phenotype.

3.5. Delayed greening phenomena

Another manifestation of delayed senescence strategy related to the phenomena of delayed greening has been studied in details (Kursar and Coley, 1992; Miyazawa et al., 1998; Miyazawa and Terashima, 2001). Kursar and Coley (1992a) found that some shade-tolerant evergreen species in a tropical rainforest invested substantial amount of nitrogen into the leaf after full leaf area expansion when the leaf became tough enough. They proposed a hypothesis that delayed greening would be an effective strategy for minimizing the loss of resources caused by the removal of young leaves by herbivory, and referred to the species showing such slow photosynthetic development as "delayed greening" species to distinguish them from "normal greening" species. Young leaves of delayed greening species were red, brown or white and these species showed periodic shoot growth (Kursar

and Coley, 1992b). These characteristics were also recognized in many evergreen broad-leaved trees in the warm-temperate forests in Japan (Miyazawa et al., 1998). Many of these species, however, grow on sunny sites (Miyazawa et al., 1998). A clear tendency that photosynthesis was delayed with the increase in leaf dry mass per unit area during maturation of leaves was found by Miyazawa et al. (1998). Based on this relationship, these authors concluded that the difference between normal greening and delayed greening species was not qualitatively distinct, but gradual. This means that delayed greening is a general phenomenon in the species having higher leaf dry mass per unit area. In addition, Miyazawa et al. (1998) pointed out that the delayed greening was not necessarily related to herbivory. Delayed greening should be used as a strategy to avoid unfavorable low temperatures during early spring (I. Terashima, personal communication).

3.6. Nitrogen remobilization and delayed senescence

Remobilization of nitrogen contained in photosynthetic proteins requires the co-ordination of several pathways for breakdown of soluble proteins, thylakoid proteins and pigments (Thomas et al., 2003). Although nitrogen contained in the chlorophyll molecules cannot be retrieved from the senescing leaf, chlorophyll degradation is required to prevent the accumulation of free chlorophyll and toxic chlorophyll catabolites (Hörtensteiner, 2004).

The delayed leaf senescence mutants of *Arabidopsis* have been used for studying the interactions between oxidative stress tolerance and longevity control in plants (Oh et al., 1997). In transgenic tobacco with delayed senescence, altered nitrogen allocation from the older leaves with reduced contents of protein and chlorophyll to the younger leaves was observed (Jordi et al., 2000). The delayed leaf senescence *ore* mutants were described by Oh et al. (1997).

3.7. Molecular mechanisms of the delayed senescence

The molecular mechanisms underlying the delayed senescence phenomenon have been also poorly investigated. Gan and Amasino (1995) described over-expression of a gene for isopentenyl transferase (IPT) under the control of a senescence-specific promoter which resulted in the auto-regulated production of cytokinins as an initial stage of delayed senescence. It was also manifested that tobacco plants expressing delayed senescence showed overreduction of the electron transport chain in old IPT leaves (Wingler et al., 2005). Although the leaves of transgenic tobacco plants with delayed senescence stayed green, photosynthetic activity did eventually decline (Jordi et al., 2000). Interestingly, some transgenic *Lolium multiflorum* plants with delayed senescence developed spontaneous lesions (Li et al., 2004), while leaves of maize plants expressing the same construct progressed directly from fully green to bleached and desiccated without intervening yellowing phase (Robson et al., 2004). These observations demonstrate that age-dependent cell death is not necessarily a consequence of senescence, but it can occur independently of the senescence process.

For induction of senescence, the accumulation of some sugars is important. In contrast to wild-type plants, the

hexokinase-1 mutant *gin2-1* did not accumulate hexoses and senescence was delayed (Pourtau et al., 2006). Induction of senescence by externally supplied glucose was partially abolished in *gin2-1*, indicating that delayed senescence was a consequence of decreased sugar sensitivity. Taken together, these results showed that *Arabidopsis* leaf senescence was induced rather than repressed by sugars (Ono et al., 2001; Yoshida, 2003).

3.8. Decapitation and delayed senescence

When a leaf is no longer required by the plant, the senescence process is induced and recycling of all mobilisable nutrients occurs. The final stage of this process is leaf death, but it is actively delayed until all nutrients have been removed through the processes of developmental senescence (Buchanan-Wolaston, 1997). This is clearly illustrated by experiments showing that senescence is reversible. A leaf that is completely yellow and has mobilized the great majority of its nutrient content can be induced to re-green by decapitation (Thomas and Donnison 2000; Zavaleta-Mancera et al., 1999; Yordanov et al., 2008a, b). The most peculiar phenomena characterizing the reversibility of senescence after decapitation are recovery of chloroplast structure, synthesis of chloroplast proteins and recovery of photosynthesis (Kutick et al., 1998).

Intact cotyledons represent an appropriate model system for studying leaf senescence as they have a short life span; they senesce gradually with the progression of seedling development and die shortly after the appearance of the differentiated leaves (Marshall and Kozlowski, 1976). The major physiological function of cotyledons is to ensure the development of the growing seedling until differentiation of photosynthetically efficient leaves. Although cotyledon senescence is not fundamentally different from leaf senescence, organ specific differences have been reported with respect to the photosynthetic activity of these two leaf organs during natural senescence (La Rocca et al. 1996).

Reversibility of senescence could be achieved artificially in cotyledons by decapitation of the entire epicotyl above the yellowing cotyledons (Mishev et al., 2005). Changes in CO_2 exchange rates, Rubisco levels and photorespiration were reported for presenescent, senescing and rejuvenated soybean cotyledons (Marek and Stewart, 1992). It was shown that during natural senescence of *Cucurbita pepo* (zucchini) cotyledons chlorophyll content decreased in parallel with the physiologically active cytokinins whereas cotyledon rejuvenation following plant decapitation correlated with increased levels of physiologically active cytokinins (Ananieva et al., 2004).

4. Decapitation of plants – a physiological approach for investigation of delayed senescence and rejuvenation

The changes in the physiological status of decapitated bean plants have been extensively studied in our laboratory (Yordanov and Popov, 1967; Popov et al., 1969; Yordanov et al., 2008a, b). Our experimental scheme involving a number of decapitation procedures leading to different responses with respect to retardation of the senescence process is

presented in Fig. 1. Our results showed that decapitation of bean plants above the primary leaves or cotyledons delayed considerably their senescence (Yordanov and Popov 1967; Popov et al., 1969). An increase in pigment content and rate of photosynthesis was observed (Popov et al., 1969). Similarly, Marek and Stewart (1992) showed that in rejuvenated soybean cotyledons total chlorophyll increased and CO_2 exchange rate recovered to the maximal rates. In addition, Rubisco activity and photorespiratory metabolism were recovered (Marek and Stewart 1992). Ananieva et al. (2004) reported that during a 10-day rejuvenation period of *Cucurbita pepo* (zucchini) cotyledons, following decapitation of the epicotyl, total chlorophyll increased almost two-fold when compared with that of the naturally senescing 5-week-old cotyledons. The process of re-greening in decapitated plants was investigated in details by Zavaleta-Mancera et al. (1999a, b). These authors reported that decapitation of *Nicotiana rustica* L. plants above a single senescent leaf induced re-greening, which was promoted by cytokinin treatment. In such a case, the process of re-greening required low light illumination. The decline in leaf protein content and increased protease activity observed during senescence were reversed upon re-greening. Western blotting showed that light-harvesting chlorophyll a/b-binding protein declined considerably during senescence, but during re-greening it increased reaching the levels in the green leaves. NADPH-protochlorophyllide oxidoreductase (POR) was present at high levels in etiolated cotyledons, but at low levels in green leaves and not at all in senescent leaves. However, POR reappeared in re-greening leaves and cytokinin accelerated its increase.

CO_2 assimilation was lowest in the non-decapitated plants in which the primary leaves were senescing whereas the highest photosynthesis was measured in the case when decapitation was applied after the emergence of the first trifoliate leaf followed by plants with primary leaves only (Yordanov et al., 2008 a,b). It was also found that if decapitation was done during different stages of bean plant ontogenesis, immediately after the appearance of the 1st, 2nd, 3rd and 4th composite leaves, the photosynthetic rate in all intact leaves was considerably decreased (Yordanov et al., 2008a, b). Our data showed that the earlier in ontogenesis decapitation was applied, the higher was the rate of photosynthesis measured in the primary leaves.

Control of leaf life span in *Phaseolus vulgaris* was modulated by epicotyl decapitation (Wilhelmova et al., 1997; Kutik et al., 1998). Senescence in decapitated bean plants was slowed down (Wilhelmova et al., 1997; Kutik et al., 1998). French bean cotyledons turned green after rising above soil surface and functioned as temporary photosynthesizing organs. Under current conditions of germination, their life span (beginning from seed imbibition) took about two weeks. Usually at the end of the second week of germination they became yellow, shrunken, and finally fell. The highest level of retardation of cotyledon or leaf senescence due to decapitation of a plant above the respective leaves was demonstrated by Huber and Newman (1976), Hudak (1981), Wilhelmova et al. (1997) and Kutik et al. (1998). It was

supposed that the degenerative changes were postponed or slowed down under the influence of the root-derived cytokinins induced by plant decapitation (Dei, 1978; Wilhelmova et al., 1997).

The decapitation procedure can activate thylakoid electron transport, thus leading to a reversal of plant senescence in the decapitated plants (Yordanov et al., 2008a, b). It is clear that the sink-source relationships which were influenced by both genotype and environmental factors, contributed to variations in photosynthesis and photoassimilates partitioning in plants.

The effect of sink-source manipulation on net photosynthesis had some relationship with the occurrence of plant senescence at the time of late wheat grain filling. The net photosynthetic rate was significantly increased by source reduction while sink reduction had a little effect on net photosynthetic rate. Source reduction accelerated plant senescence but sink reduction delayed it (Wang et al., 1998). At that time source reduction (removing one quarter of the leaves) accelerated senescence and dropped the net photosynthesis. In contrast, the limitation of such natural processes by decapitation could induce delayed senescence because of the absence of late stages of plant development.

5. Defoliation

5.1. Defoliation – negative and/or positive effects on plant fitness

Decapitated plants could be also used as an artificial model system to study defoliation (Moriondo et al., 2003; Yordanov et al., 2008b). Defoliation (leaf loss) is often described as an aspect of the herbivory effects on plant canopies (Prins and Verkaar, 1992). Defoliation is a frequently occurring phenomenon in herbaceous plants and tropical forests. Leaf area loss is a typical damage due to fungal and insect attacks or hail. Plants in tropical rainforests are often subjected to physical damage from falling canopy debris or herbivory (Prins and Verkaar, 1992 and references therein). Generally, herbivores affect negatively plant performance, but consumption of vital plant organs sometimes seems to increase plant fitness (Prins and Verkaar, 1992). Therefore, there is some controversy about the ecological importance of defoliation due mainly to the various effects of the environment on plant metabolism. For instance, arborescent, graminoid, boreal, tropical, annual, biennial, perennial plants etc. react diversely to defoliation. Seedlings of arborescent palm *Sabal palmetto* (Walt.) Lodd. ex. Schultes (cabbage palm) grow very slowly in forest understories and survive damage and defoliation well (McPherson and Williams, 1998). The authors suggested a potential importance of total non-structural carbohydrate pools in the ability of cabbage palm seedlings to recover from the loss of the above-ground tissue caused by fire, grazing, or shallow burial by storm debris. Large below-ground total non-structural carbohydrate pools in *S. palmetto* seedlings appeared to enable them to survive the most frequent defoliations (e.g., frequent grazing or mowing).

The ability of graminoids such as perennial ryegrass (*Lollium perenne,* L) to regrow after defoliation is related to the position of leaf meristem and reserves beyond the reach of animals and machines

(Berthier et al., 2009). Perennial ryegrass uses fructans (fructose polymers) as carbon storage compounds that are located mainly in leaf sheaths and in the leaf growth zone of elongating leaves. Mature grass leaves are composed of a blade and a sheath. The growing part of the elongating leaf is confined to its basal region that is enclosed by the sheaths of older leaves. Berthier et al. (2009) found an apoplastic phloem loading mechanism with transport of sucrose from leaf sheaths to the places of elongation of the new leaf. The level of *L.perenne SUcrose Transporter 1* (*LpSUT1*) expression increased in leaf sheaths in response to defoliation. On the other hand, annual grasses were more sensitive to defoliation in comparison to perennials grasses. Defoliated grass annuals such as *Avena barbata* (Poaceae) did not reach the same biomass as controls. In addition, no evidence for photosynthetic up-regulation in defoliated compared to control plants was found.

Under drought conditions, expansion in the area of new leaves following defoliation was markedly restricted and defoliation caused a significant yield reduction (Li et al., 2005). This result indicated that water management was very important for increasing defoliation tolerance of soybean. Therefore, decapitation of plants in controlled laboratory conditions can cause "pure" responses while in nature conditions many factors and fluctuations of the environment should induce various responses not always appearing as an overcompensation effect. The effects of defoliation depended on the growth stage when leaf loss occurred (Moriondo et al., 2003, Yordanov et al., 2008a, b).

5.2. Compensation effect during defoliation

Plants show different physiological responses to defoliation. Firstly, Eaton (1931) found compensatory productivity after defoliation. The compensation effect of defoliation via delayed leaf senescence and new leaf area expansion enhanced the light interception capacity of defoliated plant canopies. Owen and Wiegert (1976) suggested that overcompensation after defoliation led to increased plant fitness. Defoliated plants compensate the loss of biomass in a different manner. Overcompensation does not necessarily occur (Moriondo et al., 2003). It occurs when grazed plants show higher growth production than control plants. Equal compensation occurs when grazed and control plants have equal production while undercompensation occurs when production is lower in grazed than control plants (Belsky, 1987). Compensatory photosynthesis and the resulting compensatory growth after defoliation have been broadly reported for more than three decades (Prins and Verkaar, 1992 and references therein). Increases in photosynthesis of leaves remaining after defoliation may result either from shifts in leaf photosynthetic characteristics or from improved light penetration in the canopy (Anten and Ackerly, 2001).

5.3. Delayed senescence after defoliation

Delayed senescence in response to leaf loss and re-greening of yellow senescent leaves were firstly reported forty years ago (Woolhouse, 1967; Wareing et al., 1968). The main physiological responses to defoliation include stimulation of photosynthetic rate, altered hormonal balance, delayed plant senescence and

redistribution of carbohydrates and mineral nutrients as a result of changed sink-source relationships. Although hormones have been implicated as potential regulators of sink activity, and thus of sink mobilizing ability, little direct evidence is available to explain their role. Saftner and Wyse (1984) suggested a relatively close association between the physiological activities of ABA and IAA on the transport of sucrose into sugar beet taproot tissue. Defoliation of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) produced new bud formation that possibly involved cytokinin promoted outgrowth of lateral buds (Cline et al., 2006).

5.4. Sink-source relationships after defoliation

Defoliation induced artificially in *Nicotiana tabacum* (Kolodny-Hirsch et al., 1986) was accompanied by increased efficiency in assimilating dry matter and increased contents of chlorophyll, soluble proteins and Rubisco (Alcock, 1964). Some herbaceous perennials redistribute carbon from leaves and stem to meristems with a goal to form new leaves (Cyr and Bewley, 1990). Yordanov et al. (2008a, b) have suggested that re-growth following defoliation can be increased as a consequence of changes in the sink-source relationships.

The altered sink-source relationships in defoliated plants affect photosynthesis. Manipulating the sink-source ratio in *Prunus cerasus* trees by partial defoliation Layne and Flore (1995) investigated the phenomenon of the end-product inhibition of photosynthesis. They established that net assimilation rate of the most recently expanded source leaves of defoliated plants was significantly higher compared to non-defoliated (control) plants. Zhou and Quebedeaux (2003) monitored photosynthesis and carbohydrate metabolism in apple source leaves during a 7-day period after source-sink manipulations by partial defoliation treatments. They found that when shoots were partially defoliated, starch and glucose concentrations in the remaining source leaves declined steadily during the investigated period. Sorbitol and sucrose concentrations decreased during the first 2 days after defoliation, then increased during the following 5 days. Net photosynthesis of the remaining leaves measured at ambient and elevated CO_2 levels was also markedly enhanced. These studies suggested that metabolism of sorbitol, sucrose and starch, three photosynthetic end products in mature apple leaves, was co-ordinately regulated in source leaves in response to source-sink manipulation.

6. Decapitation and rejuvenation – hormonal balance and the role of apical dominance

6.1. Altered hormonal balance after decapitation

Gan (2007) considers that cell's life history consists of 2 processes - mitotic division and post-mitotic life pattern. On this basis, he differentiated mitotic and post-mitotic senescence in plants. A typical example of mitotic senescence is the delay in shoot apical meristem growth. Internal factors influencing senescence include age, levels of plant hormones and other growth substances, and developmental processes such as reproductive growth.

The most extensively studied endogenous factors regulating leaf

senescence are the plant growth regulators. In general, ethylene, abscisic acid (ABA), jasmonic acid (JA) and its methyl ester (MeJA), salicylic acid (SA) and brassinosteroids (BRs) promote senescence. In contrast, cytokinins (CKs), auxins, gibberellins (GAs), and polyamines are retardants of senescence. The investigation of various types of plant hormones separately is hampered due to the presence of a complex network of signalling pathways of plant hormones. Moreover, at the level of signal transduction plant hormones interact not only with each other, but also with a set of various metabolite signals and environmental factors (Beaudoin et al., 2000).

Decapitation leads to substantial changes in the biosynthesis and metabolism of plant hormones, especially auxin and CK production and transport, thus affecting the hormonal interactions in plants. It is considered that decapitation removes the dominant phloem sink and causes marked changes in sink-source relationships (Jiang et al., 2001).

6.2. Auxins

The results of Thimann and Skoog (1934) showing that exogenous auxin repressed the lateral bud outgrowth in decapitated shoots of *Vicia faba* led to the hypothesis that auxin synthesized in the shoot apex could play a role in the inhibition of axillary buds outgrowth. This evidence has given strong support for a role of auxin in apical dominance. To date auxin derived from the shoot apex and transported basipetally down the stem is recognized as the decisive regulatory signal that induces axillary buds to enter dormancy thus controlling apical dominance (Phillips, 1975; Cline, 1994 and references therein). Apical dominance is generally defined as the control exerted by a shoot apex as a dominant organ over the outgrowth of the dominated axillary buds.

6.3. Cytokinins

The correlation of increased CK levels following decapitation with reversal of the symptoms of senescence, thus leading to leaf rejuvenation was shown earlier (Mothes and Baudisch, 1958; van Staden and Carmi, 1982). CKs are known as the major senescence-inhibiting hormones (van Staden et al., 1988; Smart, 1994). The CK effect to reverse the symptoms of senescence is based on the ability of these phytohormones to activate greening of various dicotyledonous plants. A close relationship *in situ* between chloroplast ultrastructure and the content of biologically active CKs was found (Ananieva et al., 2004). Senescence can be delayed after exogenous application of CKs (van Staden et al., 1988) or due to overproduction of CKs in transgenic plants transformed by the CK biosynthesis *ipt* gene (Gan and Amasino, 1995; Jordi et al., 2000). In addition, senescence correlates with a decline in some CKs (Van Staden et al., 1988). It was earlier reported that as in leaves, the progression of senescence in *Cucurbita pepo* (zucchini) cotyledons correlated with a gradual decrease in the concentration of the biologically active CKs, especially *trans*-zeatin (Z) together with an increase of the storage CK O-glucosides (Ananieva et al., 2004). The effect of rejuvenation on CK levels was observed after a 10-d recovery period. In contrast to the senescent 5-week-old cotyledons, the rejuvenated zucchini

cotyledons had increased levels of physiologically active CKs due mainly to an increase in Z and *trans*-zeatin 9-riboside (ZR) as well as lower levels of storage CK O-glucosides. Decapitation caused an increase in xylem transported CKs before a measurable growth response of lateral buds was observed whereas application of 1-naphthylacetic acid (NAA) to the stump prevented the increase in CK levels in the xylem exudates of pea plants (Li et al., 1995). These results suggested that the elevated CK concentration participated in the release of pea lateral buds from apical dominance.

6.4. Interaction between cytokinins and auxins

Based on the model of the mutual interactions of auxin and CK in regulating correlative dominance, the process of rejuvenation observed after decapitation of plants could be explained by the increased levels of endogenous CKs due to *de novo* biosynthesis in the nodal stem together with elimination of competition from the epicotyl as a sink for CKs. In order to prevent auxin biosynthesis stimulation, axillary buds should be removed as they emerge. Thus, decapitation can serve as an approach to prolong leaf life span.

Decapitation in bean and pea plants resulted in the interruption of polar indole-3-acetic acid (IAA) transport from the apical meristems. It led to a very substantial increase in root-derived CK levels in the xylem sap or shoot (Li et al., 1995). Guenavara et al. (1995) showed that the excision of the apex of pea plants resulted in the release of inhibited lateral buds from apical dominance. This could be entirely prevented by applying NAA to the cut end of the shoot. Removal of the apex resulted also in a rapid and rather large increase in the endogenous concentrations of ZR, isopentenyladenosine (iPA) and as yet unidentified polar zeatin derivative in the node and internode below the point of decapitation. The accumulation of ZR and iPA was strongly reduced by the application of NAA. The authors suggested a mutual interaction between the basipolar IAA transport system and CKs obviously produced in the roots and transported via the xylem into the stem of the pea plants. It was earlier reported that decapitation of pea plants on the 7th day after seed germination above the second node resulted in a 2-3-fold decrease in the IAA level in the internodes while the CK level increased 56 times for Z and ZR and 1.5-2-fold for isopentenyladenine (iP) and iPA (Kotova et al., 2004). In contrast to internodes, the iP and iPA contents in the roots of the decapitated seedlings did not change, but the levels of Z and ZR increased 1.5-2-fold compared to intact plant roots. The IAA level in the apical region of the root remained almost unchanged after the removal of the shoot apex. The authors concluded that the apical meristem of the main root was not the site of the CK response to the auxin signal coming from the stem apex and that the slight accumulation of Z and ZR after decapitation was due to the upper zones of the root.

Thus, whereas apically produced auxins down-regulate CKs in the roots, root produced CKs up-regulate auxins in lateral and terminal buds, suggesting a strong mutual interaction between these two hormones, which may be important in regulating the balance between root and shoot growth (Bangerth et al., 2000). Molecular biology has also confirmed the

importance of auxin and CKs in apical dominance (Cline, 1994). It has recently been shown, however, that CK that promotes axillary bud outgrowth after decapitation is locally biosynthesized *de novo* in the nodal stem and not transported from the roots (Tanaka et al., 2006; Sato et al., 2009). It was demonstrated that auxin derived from the shoot apex suppressed local biosynthesis of CKs in the nodal stem through down-regulation of the expression of *PsIPT2,* a member of the *IPT* family. Decapitation led to depletion of auxin in the nodal stem which immediately induced *PsIPT2* expression thus leading to increased CK levels (Tanaka et al., 2006). In addition to suppression of CK biosynthesis, auxin derived from the shoot apex might regulate CK levels in the stem by inducing CK degradation through the regulation of *CKX* expression thus controlling the activity of cytokinin oxidase/dehydrogenase (CKX) which is a key enzyme in CK degradation (Sato et al., 2009). These authors have proposed a model of interactions between auxin and CK to control shoot branching through a complex cross-talk in which hormones affect the expression of genes related to both biosynthesis and metabolism.

It is clearly documented that auxin can regulate CK pool sizes and vice versa (Nordström et al., 2004). Data demonstrating that CK or auxin overproduction in transgenic tobacco plants decreased the pool size of the other hormone illustrate the interaction of the two hormones at the metabolic level. It was shown that auxin mediated a very rapid negative control of the CK pool by suppressing its biosynthesis in *Arabidopsis* (Nordström et al., 2004). Thus, auxin directly affected CK biosynthesis by regulating *IPT* expression which is involved in the first step of CK biosynthetic pathway.

Although the intimate mutual interrelationship between the promotive CKs in the stem and inhibitory shoot apex-sourced auxin is central to all current models on axillary bud dormancy release, interactions with other hormone groups have also been considered. Chen et al., (1997) determined endogenous levels of IAA, CKs, gibberellins (GAs) and abscisic acid (ABA) in *Rhododendron obtusum* plants, which had been decapitated. It was found that decapitation could significantly decrease the level of IAA in the shoot segment. The decapitation could also elicit an increase of GA levels and a decrease of ABA level in the shoot segment and the terminal bud (Roland et al., 2006). The excision of the apex of pea plants resulted in the release of inhibited lateral buds from apical dominance. Removal of the apex also resulted in a rapid and rather large increase in the endogenous concentrations of CKs.

6.5. Gibberellins

Gibberellins are diterpenes that promote stem and leaf growth (Schippers et al., 2007). GAs retarded senescence of excised leaf tissue from *Taraxacum oficinale* by maintaining chlorophyll levels and RNA synthesis (Fletcher and Osborn, 1965). Even when chlorophyll and protein loss was halfway complete, addition of GA blocked further degradation. A study performed on the leaves of romaine lettuce showed a clear age-related decline in GA levels and absence in senescing leaves (Schippers et al., 2007). Wolband and Ross (2001) showed that auxin was necessary for normal GA biosynthesis in

stems of tobacco. Decapitation of tobacco (*Nicotiana tabacum* L.) plants reduced the endogenous levels of IAA, GA_{20}, and GA_1 (the bioactive GA), in the internode tissue below the excision site. Application of IAA to the stump of decapitated plants dramatically increased GA_{20} and GA_1 content.

6.6. Ethylene

Ethylene is the key hormone in regulating the onset of leaf senescence (Schippers et al., 2007). The senescence-delaying hormones auxin and CK stimulated ethylene production in lettuce leaves (*Lactuca sativa* L.), which might account for their limited stay-green properties. Changes in ethylene level after decapitation and defoliation were reported (Wilhelmova et al., 2004). Ethylene production increased at the end of cotyledon life span in non-decapitated bean plants. However, in the decapitated plants this increase started earlier, proceeded more slowly, and did not reach its maximum at the time of abscission (Wilhelmova et al., 2004). Such an increase in ethylene production could be induced by wounding due to the removal of leaves (O'Donnell, 1996).

6.7. Involvement of other hormones and plant growth regulators in the senescence program and defoliation/ decapitation plant responses

In vegetative tissues, ABA plays a role in the response to drought to prevent water loss by stomatal closure and the maintenance of vegetative growth by inhibiting the transition to reproductive growth. Under nonstressful conditions, ABA in plant cells is maintained at low levels since ABA inhibits plant growth. In vegetative tissues, ABA levels increase during drought, salt and cold stress (Schippers et al., 2007). Gocal et al. (1991) investigated the early changes in the concentrations of IAA and ABA in the larger axillary bud of 2-week-old *Phaseolus vulgaris* seedlings after removal of the dominant apical bud. They observed, within 4 h after decapitation, a 5-fold increase of IAA concentration in the axillary bud, remaining relatively constant thereafter. The concentration of ABA in the axillary buds of decapitated plants was by 30 to 70% lower than in the buds of intact plants. Anatomical assessment of the larger axilliary buds 8 and 24 h following decapitation showed that most of the growth was due to cell expansion, especially in the internodal region. These results demonstrated that following decapitation, coincidental with the rise in IAA concentration in the axillary buds was a modest, but significant reduction in ABA concentration.

Brassinosteroids (BRs), polyhydroxylated steroid hormones, regulate the growth and differentiation of plants throughout their life cycle (Schippers et al., 2007). The induction of senescence by BRs might be mediated through reactive oxygen species (ROS). Interestingly, BRs can induce ethylene biosynthesis genes in mung bean (see Schippers and references therein). At present, no evidence for the participation of brassinosteroids in defoliation/decapitation plant responses is available.

The promotional effect of methyl ester of jasmonic acid (MeJA) on senescence progression was firstly shown after application to detached oat leaves (Ueda and Kato, 1980). Exogenously applied JA or MeJA resulted in a decreased expression

of photosynthesis-related genes like Rubisco. We have recently shown that compared with darkness, MeJA is a more potent inducer of senescence in zucchini cotyledons based on the stronger decrease in chlorophyll content, photosynthetic rate and chloroplast transcriptional activity (Ananieva et al., 2007). The higher capacity of MeJA to promote cotyledon senescence is at least partly due to the stronger reduction in the levels of biologically active CKs, especially *trans*-Z.

Accumulation of MeJA was observed after herbivore attack in plants which is associated with the induction of defence responses that can benefit fitness, but are costly to express (Cipollini, 2007). The consequences of the overexpression of MeJA on plant tolerance to defoliation, plant competitive effect and response, were studied using *Arabidopsis thaliana* MeJA overexpressing genotype (Cipollini, 2007). Defoliation reduced height more strongly in the wild-type than in plants overexpressing MeJA. In natural plant populations, overexpression of MeJA-mediated responses should enhance the resistance to herbivores, pathogens and competitors, but it is in expense of the plant fitness and probably constrains plasticity in response to changing environmental conditions.

Salicylic acid (SA) is a phenolic compound that has been identified as a key signaling molecule in various plant responses to stress like pathogen invasion, exposure to ozone and UV-B (Schippers et al., 2007). A role of SA during later stages of the senescence program has been supposed. A possible relationship between SA levels in defoliated Arabidpsis plants and pathogen defense in these plants has been suggested (Barto and Cipollini, 2005).

7. Photosynthesis in decapitated plants as a consequence of the overcompensation effect and its eco-physiological role for defoliated plants

The compensatory productivity enables defoliated plants to replace tissue lost due to herbivores (Eaton, 1931). The effect of overcompensation due to defoliation leading to increased plant fitness was proposed by Owen and Wiegert (1976). They showed that plants subjected to herbivory actually increased their production. Van der Meijden (1990) considers herbivory as a trigger growth of the grazed plants.

Most of the studies devoted to the physiological responses to defoliation/ decapitation have shown a rise in photosynthesis or a changed allocation pattern. Possibly, overcompensation depends on these processes. The changes in thylakoid electron transport could be considered as an important parameter characterizing the overcompensation effect (Yordanov et al., 2008a, b).

Changes in chlorophyll fluorescence and millisecond-delayed fluorescence were recently investigated as a measure of the alterations in PS2 electron transport during senescence and rejuvenation of decapitated bean plants (Fig. 1; Yordanov et al., 2008a, b). Analysis of the OKJIP transients based on the JIP-test (Strasser et al., 2004) showed an increase in several biophysical parameters of PS2 in decapitated plants, more specifically, density of active reaction centers on a chlorophyll basis, trapping efficiency, electron transport rate and performance

index (Yordanov et al., 2008a). In contrast, a decrease in the absorbed light energy per reaction center was observed (Yordanov et al., 2008a). Such a decrease in light absorption could be the result of the PS2 down-regulation that appeared as an increase in Q_B-nonreducing PS2 centers. The effect was similar when all leaves except the primary leaves were removed (D_0 variant in Fig. 1). In the defoliated plants with preserved apical buds (variant Df in Fig. 1) values similar to those of the decapitated plants with primary leaves only (D_0) were obtained. In these plants, the PS1-driven electron transport was accelerated, and the size of the plastoquinone pool was enhanced. Our results showed that decapitation can retard the senescence of primary leaves, expand leaf life span and cause activation of both PS1 and PS2 electron transport.

The decapitation procedure showed similarities with the process of defoliation. The overcompensation effect occurring after defoliation could initially be manifested as acceleration of the linear photosynthetic electron flow in the rest of the leaves. A decrease in the yield of delayed fluorescence and changes in DF thermograms implied acceleration of the electron transport beyond PS2 in the decapitated plants (Yordanov et al., 2008b). Consequently, the decapitation procedure can activate thylakoid electron transport thus leading to a reversal of plant senescence in the decapitated plants. It is suggested that modulated sink-source relationships in the decapitated plants could be involved in the changes of the studied photosynthetic parameters (Yordanov et al., 2008a, b).

The overcompensation effect can lead

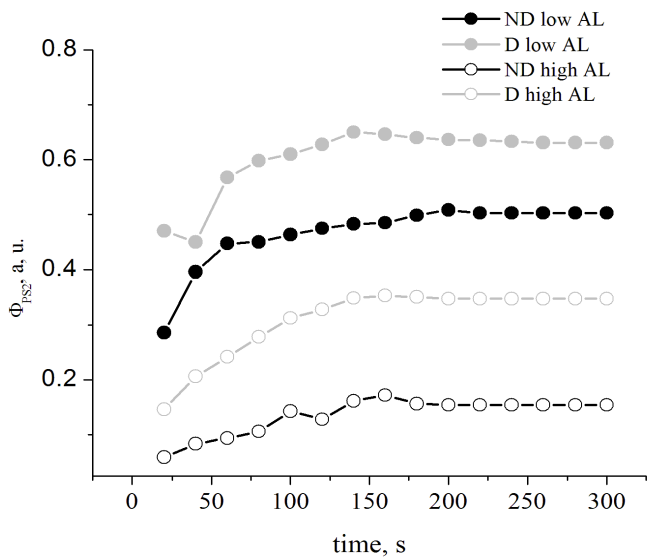

Fig. 2. Induction kinetics of the actual PSII quantum yield during dark/light transitions in control, non-decapitated, ND (black symbols and lines) and decapitated, D_0 (grey symbols and lines) 21-day-old bean plants measured at actinic light intensities of 90 μmol m^{-2} s^{-2} PFD (closed symbols) and 980 μmol m^{-2} s^{-2} PFD (open symbols). Curves for one sample per variant are shown. The remaining samples (n = 4 per variant) deviated less than 5% from the reproduced sample.

to better adaptation to the unfavourable environmental conditions. For example, changes in chlorophyll *a* fluorescence in the leaves of decapitated plants indicated better adaptation to higher actinic light intensities (Fig. 2). The induction of the actual PS2 photochemical activity, Φ_{PSII} showed significantly higher values in the leaves of 21-day-old decapitated bean plants (Fig. 2). This increase was independent of the actinic light intensity. As shown in Fig. 2 the decrease in Φ_{PSII} was expressed to a higher extent in control plants (variant ND) at higher actinic light intensities. In addition, an enhancement of PSII "photochemistry" in decapitated plants was observed. The reduced Φ_{PSII} values measured in the control plants suggested higher capacity for photoinhibition. Similar resistance of photosynthesis was found in decapitated tomato plants subjected to drought stress (Stefanov, unpublished results), whole bean plants subjected to prolonged dark treatment or individually darkened bean leaves (Yordanov, unpublished results). Higher resistance of decapitated plants to various stresses such as high temperature (Yordanov and Weis 1974) could imply that leaf physiology can be switched to limitation of the unfavourable environmental effects. Thus, damaged shoot structure after decapitation/ defoliation can be compensated by

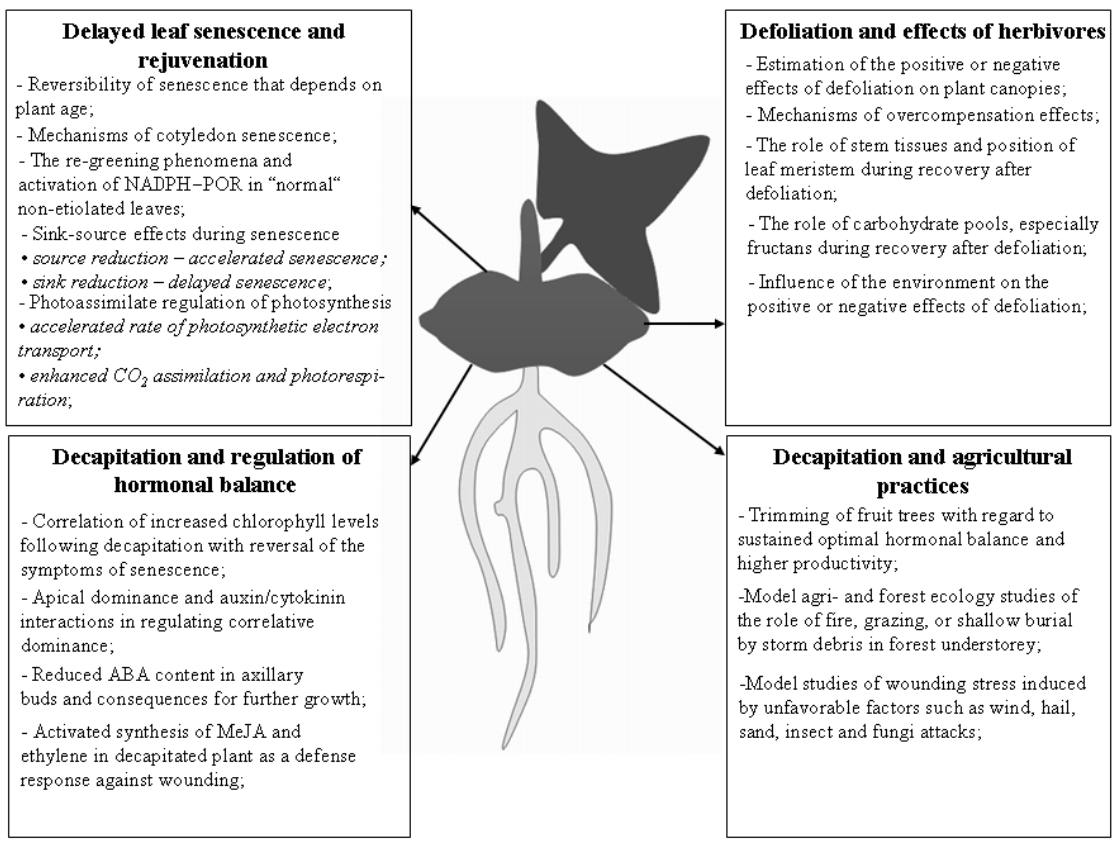

Fig. 3. Involvement of the decapitation approach in plant physiology investigations and agricultural practices.

more flexible adjustment to altered environmental conditions.

In conclusion, using the decapitation procedure leaf senescence can be reversed and leaf life span is prolonged. This approach can be effectively applied in plant physiology investigations and various agricultural practices (Fig. 3). Thus, decapitation could serve as a useful physiological approach to study control mechanisms of leaf senescence and its reversibility as well as to search for more effective agricultural practices including trimming of fruit trees related to their productivity, herbivory and fire influences on plant canopies, wounding-related stress effects such as wind, hail or sand.

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