

Chapter 11

Adaptation Mechanisms of *Pinus sylvestris* L. in Industrial Areas

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11.1 Introduction

In spite of a positive direction of changes occurring in the contaminated environment, the local industry still appears to exert a negative influence on plant vegetation. Forests which grow in many highly industrialized zones enable research on the influence of anthropopression on the natural population and are one of the best models for the study of plant adaptation to heavy metals in soil. In some cases, it is possible to follow processes of re-naturalization occurring on post-industrial areas in situ. Adaptive genetic diversity reflects differences in the survival capabilities of individuals exposed to stress and shows the selective pressure against trees with specific genotypes. This chapter emphasizes on the Scots pine (*Pinus sylvestris* L.) as one of the most frequently used bioindicators in the European forests and their application in the study of microevolutionary processes in tree populations. It may enhance a better understanding of how the soil pollution can change the genetic structure of important forest species.

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11.2 *Pinus sylvestris* as a Bioindication of Tree Species

A critical role in the adaptive potential of a species plays the presence of substantial heritable genetic variation. The evolutionary response of a population to a changing environment is the intensity of ‘the best’ phenotype (Rice and Emery 2003). Thus, looking for an adaptation, the following questions arise: (1) what kind of features characterize the most invulnerable individuals, (2) is it reflected in the structure of the population, and (3) is it a matter of a special adaptation process or just a matter of chance?

One of the best models for the study of adaptation to the industrial areas is that associated with the impact of heavy metals (HM). According to multiple sources (Linhart and Grant 1996; Markert et al. 2012), only a complex approach to the problems connected with the adaptation of living organisms to a highly polluted environment allows a better understanding of evolutionary mechanisms developed by the observed individuals. Despite many years of research, still very little is known about the mechanisms of conifer response to heavy metal toxicity.

In nature, the process of selection may lead to distinct differences between resistant and sensitive individuals and, finally, create genetic differentiation with respect to metal tolerance. Different concepts of plant adaptation in stressful environments can be found in many articles (e.g. Dickinson et al. 1991; Godbold 1998; Kozłowski and Pallardy 2002; Kozlov et al. 2009). Such a wide range of tolerance patterns has been reflected in comprehensive studies conducted using methods of statistical meta-analysis. Surprisingly, only two regularities were registered concerning the effects of pollution (including contamination by HMs)—namely, (1) the diversity of organisms with respect to decreases in size due to a strong dose of pollutants and (2) larger plants (trees) suffered more from pollution than smaller (herbaceous) plants (Kozlov et al. 2009; Kozlov and Zvereva 2011). In the case of trees, where heritable changes take place at a slow rate, selection for individuals with a higher tolerance may take many decades before a sufficient resistant population is built up. Thus it has been suggested that only phenotypic plasticity may provide a mechanism that improves the survival of the long-lived trees in metal-contaminated environments (Dickinson et al. 1991; Korshikov et al. 2002).

A positive direction of changes occurring in the environment is observed in many highly industrialized zones. Despite this, the local industry is still appearing to exert a negative influence on forest areas. Upper Silesia (South Poland) is a region where a massive extinction of forests, particularly affecting the pine ecosystems, was observed in the early 1980s. Currently, natural regeneration of forests in this region enables research on the influence of anthropoppression on the Scots pine population. In this case, it is possible to follow processes of re-naturalization occurring on post-industrial areas in situ. Research already undertaken in this region showed an interesting phenomenon of differentiation among the Scots pine populations with respect to health status. In a homogeneous age group, there are two types of trees: gradually dying, with distinct damage, and healthy, with no signs

of damage. Observed differences affect the ability to pass genetic material to next generations and the process of natural selection can induce reproductive success as a measure of competitive effectiveness. The ongoing transformation of tree populations is a classic example of local adaptation.

Simultaneous verification of the number of different parameters allows more precise diagnoses of tree fitness and indicates with the highest probability in which stress factor is the most responsible for the occurrence of visible damage. The complexity of responsiveness of an organism to stress is the reason that a combination of the specific type of stress with its particular result in terms of cause and effect is often very difficult. The study of species used in bioindication is particularly important in this context due to large amounts of standardized data available on the topic.

Bioindicators are defined as organisms which carry information about the quality of the environment. Trees are considered sensors that record the environmental disturbances, as they live for a long time in the same place and are widespread geographically. Conifers capture pollutants from the atmosphere and deposit them directly on the bark and needles or take them up from the soil by the roots. Generally, coniferous species have much higher heavy metal content than deciduous species. *Pinus sylvestris* as a species very sensitive to industrial pollution is frequently used for monitoring contamination by HM (Nieminen and Helmisaari 1996; Derome and Saarsalmi 1999; Harju et al. 2002; Diatta et al. 2011; Ivanov et al. 2011). It is one of the most common native forests species in Europe and fulfils a series of conditions which make it useful for bioindication. The bioindicative properties of *P. sylvestris*, such as its wide geographical range, easily recognizable taxonomic range and low tolerance to toxic agents expressed in a simple-to-analyse reaction, allow not only a detailed description of the current health of forest but also identification of the direction of changes which affect them. In *P. sylvestris*, some metals, especially Pb, are accumulated in roots, probably due to physiological barriers against metal transport to aerial parts, while others, such as Cd, are easily transported (Kabata-Pendias 2004). Heavy metals enter the plant mainly by roots where they are bound to the carboxy groups of mucilage uronic acids. Close to the pollution source, concentrations of HM in all the pine compartments are higher as compared to their background levels. In conditions of severe threat by industrial emissions, pines are characterized by an unusual dwarf habit. The reduction in shoot growth and its thickness leads to changes in crown conformation. Branches and, especially, stem bark are usually richer in heavy metals than other tissues and, in addition to wood, contributed significantly to the accumulation of metals in the biomass of the polluted pine forests. However, most often in bioindication, pine needles are used.

11.2.1 *Pinus sylvestris: Morphological Alteration and Heavy Metals*

Like most coniferous trees, the Scots pine holds needles for several growing seasons, which allows for long-term observations. Taking into account that the first effects of pollution in pine stands are changes connected with leaf degradation, morphology and anatomy of needles are frequently used as a diagnostic tool for the identification of pollution. Research carried out in Upper Silesia identified several dissimilarities between the needles of trees growing under conditions of high stress and needles of trees from an ecologically clean area.

The changes in morphological and anatomical characteristics found in Scots pine needles can be a result of an exposure to elevated levels of HMs in the soil. The structure of leaves differed in relation to the control population with respect to a significant decrease in needle length correlated with an increase in needle height. Needles from the contaminated area are distinctly shorter and thicker. This is consistent with reports which showed that the reduction in leaf growth under conditions of heavy metal pollution is an expression of adaptation, because it reduces transpiration and thus limits the uptake of metals (Potters et al. 2007). The higher pollutant accumulation is correlated with the density of stomata on needles close to the smelter, and an increase of stomatal density is noted on both adaxial and abaxial needle surfaces (Chudzińska and Urbaniak 2008). The number of resin canals, another basic feature counted during pine needle observation (Lin et al. 2001), is regarded as one of the better characteristics which allow one to distinguish between resistant and sensitive trees growing in conditions of severe contamination. Alterations of the resin–canal system are closely linked to various types of stress. Damaged trees in our results show a low number of resin canals (Fig. 11.1c).

Some of the studied needle features are associated with higher resistance to the adverse conditions. Resistant trees had longer needles with a greater density of stomata and significantly more resin canals (Fig. 11.1a-c). Many authors emphasize the dependence of variation in needle area on growing conditions and explain the reduction in these parameters with the adverse effects of heavy metals on cell elongation and expansion, microtubule formation and reduction of water content in tissues (Novikova and Milyutin 2006 and literature cited herein). Therefore, longer needles have better adaptation to the high level of HMs in the soil. Low concentrations of heavy metals in leaves can cause an increase in the number of stomata resulting from the reduction of leaf area (Klánová et al. 2009; Prasad and Hagemeyer 2004). In contrast, a high concentration of metals may lead to the reduction in the number of stomata, which was detected in sensitive trees (Chudzińska and Urbaniak 2008). Our results show also that trees with better fitness have more resin canals than trees that are close to dying.

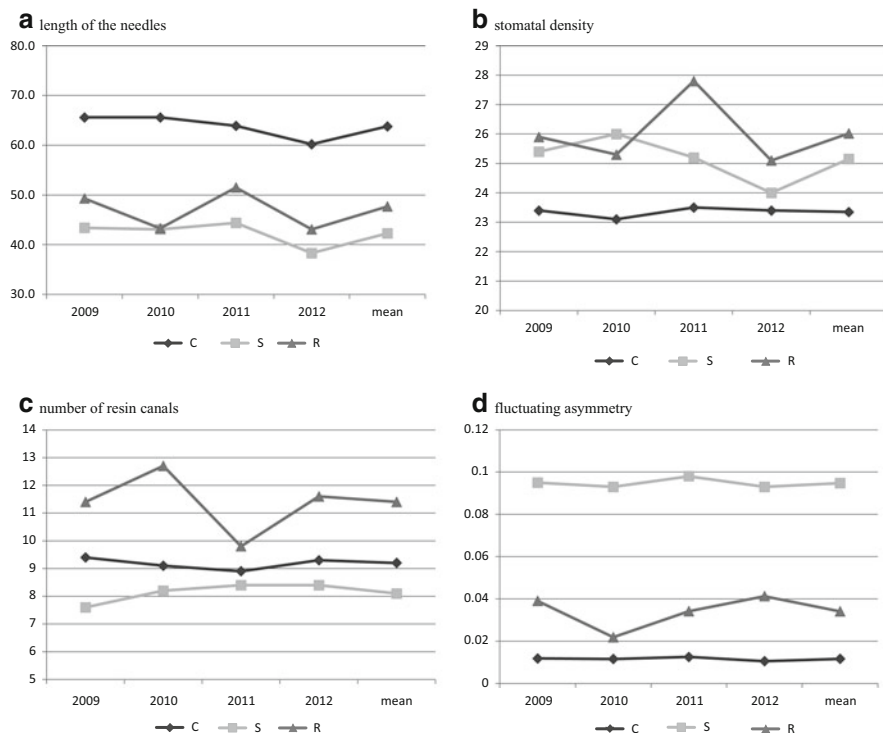


Fig. 11.1 Differences related to morphological features of *P. sylvestris* needles; (a) length of the needles, (b) stomatal density, (c) number of resin canals, (d) fluctuating asymmetry. Needles of trees from resistant (R) and sensitive (S) groups exposed to HM contamination and needles of trees from an unpolluted area (C)

Double-needed fascicles of *P. sylvestris* are additionally an ideal object for testing fluctuating asymmetry (FA), one of the parameters demonstrating an inability to control development under genetic and environmental stress (Kozlov et al. 2002; Chudzińska et al. 2013a, b). Deviation from needle symmetry as a result of disruption to normal growth may be caused by the allocation of resources from developmental quality to growth in morphogenesis under stress. Therefore, a significant increase in the value of the FA is commonly observed among the pine trees growing in the environment heavily contaminated with HMs as in Upper Silesia where the higher fitness of trees resistant to contamination was confirmed by the results of the FA measurements (Fig. 11.1d). For the two-needle Scots pine, FA measurement gives a simple answer to whether the tree is growing in unfavourable term because the variation among pine needles within a fascicle is analogous to variation between right and left sides of a bilaterally symmetrical trait (Chudzińska et al. 2013a, b).

11.2.2 *Pinus sylvestris*: Cytology and Reproduction

Disturbances occurring under the influence of HMs in cell divisions are another important parameter of bioindication. This negative impact of industrial emissions on the Scots pine lowers yields and quality of seeds (Oleksyn et al. 1994). Palowski (2000) found considerable accumulation of heavy metals in pine seeds which significantly reduced the ability and energy of seed germination in the vicinity of 'Huta Katowice' smelter. The elevated level of HMs in the soil causes an increasing number of abnormally germinating seeds, which is associated with its high mortality.

In Upper Silesia faster germination of seeds belonging to the group vulnerable to pollution than in group of resistant tree seeds was noted, and percentage of germinated seeds differ significantly (averaged 63.02 % for resistant, 46.32 % for sensitive and 90.17 % for control tree seeds). One of the reasons for these differences may be a low resistance of Scots pine seedlings to elevated concentrations of zinc, the metal present in the highest concentration in the studied area (Miasteczko Śląskie). In experimental conditions, Zn influence was manifested in reduced seed germinability, an inhibition of root growth, root development and numerous cytological aberrations (Ivanov et al. 2011). The level of Scots pine tolerance to an elevated zinc concentration is lower than that of commonly used model plants: *Arabidopsis thaliana*, *Nicotiana tabacum*, *Brassica napus* and others (Ivanov et al. 2011).

Similarly, the presence of Pb, Cu and Cd in soil induces several abnormalities as aggregation of chromosomes during metaphases, production of binucleate cells and stickiness, which increase with increasing levels of those metals (Kim et al. 2003). The increased level of aberration frequency in meristematic cells of specimens from the polluted region, which differ significantly from those recorded in the control population, indicates that the majority of these irregularities result from the impact of HM contamination on the tree stands studied. There may be various reasons for this result. First of all, the complex life cycle of conifers and the long duration of the generative phase cause a strong sensitivity of the reproductive organs to the harmful effects of a wide range of pollutants. During such a long period of maturation, seeds can accumulate numerous clearly visible DNA damages, which can be observed in their meristematic tissues. After germination, seedling roots are in the place of first contact with contaminated soil and are exposed to the highest concentrations of HMs. A lot of plants prevent or reduce uptake into root cells by restricting metal ions to the apoplast, binding them to the cell wall or to cellular exudates, or by inhibiting long-distance transport. The major role of root exudates is to chelate metals and to prevent their uptake inside the cell (Marschner 1995). The cell wall plays a key role in the immobilization of heavy metal ions and prevents their uptake into the cytosol (Manara 2012). Pine seedlings inoculated with metal-tolerant ecotypes of ectomycorrhizal fungi *Suillus* have lower metal concentrations in their needles than seedlings inoculated with sensitive strains, indicating that the metal-tolerant isolates restrict metal transfer more effectively. The influence of Zn

and Cu causes a reduction in the number of lateral roots in pine seedlings. High concentrations of both metals, along with lead, can completely destroy protoplast cells of the rootcap meristem zone, by inhibiting mitotic divisions, as a result of the damage to cytokinesis (Schützendübel et al. 2001).

In general, coniferous species do not exhibit a high protective ability against contaminants taken up from the atmosphere, and an assessment of cytogenetic anomalies in the intercalary meristem of young needles appears to be a promising test system. However, in the case of HM soil contamination, it seems to be less convenient. In fact, for all analysed specimens, a greater number of abnormal cell divisions were observed in the root apical meristems than in the meristems of needles. Chemical analysis of pine seedlings (Diatta et al. 2008) showed that the content of HMs in roots was significantly higher than in shoots.

The most commonly used indicator of stress—mitotic index (MI)—in all cases also pointed to a higher number of cell divisions in the root apical meristems than in the meristems of pine needles. The differences of the mitotic index (MI) between the seedlings from the Miasteczko Śląskie and from the NPW can be considered an adaptation, giving organisms under extreme conditions more time for repair (Prus-Głowacki et al. 2006). This was verified by the dominance of cells in prophase, when ‘arrested’ mitosis gives an opportunity to repair genomic damages, in Scots pines from the Khrenovskoy stand (Butorina et al. 2001). The highest MI observed in the meristematic cells of seedling roots from the control trees seems to confirm this suggestion.

DNA damage observed in the form of chromosomal aberrations mainly related to the first phase of mitosis. In Scots pine, we observed mainly such abnormal mitosis types as: asymmetric mitosis, chromosomal stickiness, chromosome bridges, chromosome lagging, fragments of chromosomes and retarded chromosomes. These abnormalities result from spontaneous mutations induced by HMs, while in Russia (Voronezh), similar types of mitotic abnormalities were apparently related to a high level of gaseous air pollution (Butorina et al. 2001). This indicates that, like evidence from Voronezh, soil from MŚL also contains hazardous substances capable of disturbing mitotic divisions. Interesting data obtained in studies of *Syringa vulgaris* L. and *Armeniaca vulgaris* Lam. demonstrated that the increasing yield of abnormal mitoses was related to contamination with heavy metals from the soil in which the affected populations of these plants grew (in control samples there were no such aberrations). Similarly, a broad spectrum of cytogenetic aberrations was detected in three species of the genus *Pinus* (including *P. sylvestris*) growing in the vicinity of two integrated metallurgical works in Slovakia (Mičieta and Murín 1998). According to Kalashnik (2008), HMs rarely have a direct effect on DNA, but they are strong inducers of various mitotic abnormalities. Current data confirm that heavy metal soil pollution strongly affects Scots pine populations from the Miasteczko Śląskie region, and noted differences in the fitness of specimens are correlated with the level of cytological disturbances, especially with abnormal mitosis indices. The stand includes trees resistant and sensitive to growth conditions which vary in their range of chromosomal aberrations. The increased frequencies of abnormal mitoses in cells of *P. sylvestris* from polluted plots, which significantly

differ from those recorded in control plots, indicate that the majority of these aberrations result from the impact of industrial pollution on the tree stands studied.

Mostly the chromosome aberrations are fixed by the reparative system of the cell, while the remaining defects lead to the formation of micronuclei. The formation of micronuclei results from chromosome lagging during mitosis and from multipolar mitoses. Therefore, micronuclei reflect disturbances in the genetic apparatus and are used to assess cytotoxicity and genotoxicity. For example, in the seedlings grown from the seeds collected in radioactively contaminated zones, its level was significantly elevated (Oficerov and Igonina 2008). Also the nucleolar activity (NA) is used in biomonitoring, because nucleoli are responsible for RNA synthesis in the cell, and the proportion of interphase cells with a specified number of nucleoli is an index of cell metabolism. Cytological data from multiple coniferous species suggests that pines can have many nucleolar chromosomes in their genomes. The NA is highly variable; therefore, *P. sylvestris* cells may contain from 1 to 15 nucleoli. Their number is higher under extreme conditions, e.g. in a pine growing in a bog (Chudzińska 2013). The number of nucleoli counted in silver-stained interphase pine-seedling cells varied from 1 to 15 in data from Miasteczko Śląskie. Cells with three to six nucleoli correspond to the three pairs of chromosomes with functioning nucleolar organizers, which prevailed in all studied samples. The nucleolar activity according to literature (Butorina et al. 2001; Butorina and Vostrikova 2006) is the most sensitive criterion of cytogenetic monitoring as an expression of the transcription activity of ribosomes. The number of nucleoli in a cell indicates the presence of ribosomal genes. If so, the increase of their number in trees from contaminated regions can be possibly regarded as response to stress induced by pollution of the environment. The increase nucleoli number in interphase cells and the decrease nucleolus–nucleus ratio under stressful conditions of growth have been marked in coniferous trees by Sedelnikova and Muratova (Sedelnikova and Muratova 2001). In the opinion of the authors, this phenomenon is an adaptive sign of coniferous species (as well as *P. sylvestris*) to stressful conditions. The number of nucleoli (up to 12 per cell) increases in conditions of severe stress, such as high background radiation, air pollution or soil contamination with HMs (Oficerov and Igonina and literature cited herein).

The level of chromosome aberrations, nucleolar activity and mitotic index studied in the seed and needle meristems of three Scots pine populations growing in areas differing in the degree of soil pollution provide evidence for the adverse effect of environmental stress on the chromosomal structure of these trees. With respect to environmental quality assessment, the Scots pine turned out to be a sensitive indicator species, and the analysis of chromosome aberrations in anaphase–telophase cells was most effective among the cytogenetic methods used. The differences observed between the trees growing in the same conditions cannot be explained by the influence of the environment. Their source must be sought in the varying capability of single individuals to adapt.

11.3 Sensitivity and Resistance: Genetic Basis

Organisms adapt to their environments by developing heritable tolerance mechanisms. According to Macnair et al. (2000), tolerance to heavy metals in plants may be defined as the ability to survive in a soil that is toxic to other plants and is manifested by an interaction between a genotype and its environment. Metal tolerance is a complex process by which populations respond to long-term environmental stresses by permanent genetic or epigenetic changes (Kovalchuk et al. 2004; Mehes-Smith et al. 2013). Adaptability is composed of two components: the physiological adaptability of individual trees (depending on the individual genotype) and the genetic adaptability of tree populations (based on the genetic variation within populations), which represents the evolutionary potential (Bergmann and Hosius 1996).

11.3.1 Protein Build-Up and Metabolism Control

Plants have evolved detoxification mechanisms, and consequently metal tolerance, to minimize heavy metal exposure and impact. In the cell, metals are under mechanisms of storage and detoxification strategies including transport, chelation and sequestration in the vacuole. Several types of proteins are involved in the metal chelation and in their root-to-shoot transport (Rausser 1999). The best known is the role of phytochelatins (PCs) and metallothioneins (MTs). The first (PCs) are small, heavy metal-binding, cysteine-rich polypeptides. They form complexes with toxic metal ions in the cytosol and then transport them into the vacuole, protecting plants from the deleterious effect of heavy metals (Salt and Rausser 1995). Glutathione (GSH), low-molecular-weight thiol, is a substrate for phytochelatin synthesis catalysed by the enzyme phytochelatin synthase (PCS) (Freeman et al. 2004). It is commonly believed that glutathione has a fundamental significance in determining plants' heavy metal tolerance (Yadav 2010). The second—metallothioneins (MTs)—are low-molecular-weight proteins which contain cysteine-rich domains at the amino- and carboxy-terminal regions. As they contain mercaptide groups, they are able to bind metal ions (Manara 2012). Transcription of MTs is typically induced by the same metal ion that binds to the protein, thus providing a direct activation of their protective function (Waalkes and Goering 1990). Some researches hypothesize that metallothioneins can protect plants and their DNA against oxidative damage by removing free radicals (Xue et al. 2009).

Other proteins are involved in the activation of oxidative stress mechanisms. When the concentration of heavy metal ions in the cell exceeds defence mechanisms, then the plant suffers oxidative stress due to the inhibition of metal-dependent antioxidant enzymes and production of reactive oxygen species (ROS) (Schützendübel and Polle 2002). They are very reactive molecules with an unpaired electron which can provoke oxidation and modification of cellular amino acids,

proteins, membrane lipids and DNA (Ogawa and Iwabuchi 2001). The very important role glutathione plays in the mechanisms of heavy metal stress tolerance involved detoxifying ROS through ascorbate–glutathione cycle (Yadav 2010).

Plants activate their stress responses, including the induction of many enzymes, which may have different variants called allozymes (because they are encoded by different *alleles* at an enzyme gene locus). Studies (e.g. Prus-Głowacki et al. 2006; Chudzińska et al. 2013a, b) demonstrate differential survival and reproduction of individuals with different allozyme genotypes living under stress conditions. These results would imply that allozymes may be direct sites of action for toxicity to certain toxicants and in particular heavy metals (Van Straalen and Timmermans 2002). One of the mechanisms suggested explaining the relationship between pollution tolerance and allozyme genotype was that heavy metals competitively inhibit magnesium-dependent allozymes differentially (Guttman 1994). It is suspected that the degree of resistance to a highly contaminated environment may be linked to enzyme systems involved in various metabolic pathways, whose activity is modified under the influence of heavy metals. For example, enzyme 6-phosphogluconate dehydrogenase (6PGD) plays a critical role in the oxidative pentose pathway, which is physiologically very important under stressful conditions (Bergmann and Hosius 1996). A significant role for the 6PGD was also emphasized by these authors in the adaptation of Norway spruce to heavy metal-contaminated soils. Shikimate dehydrogenase (ShDH) is connected to detoxification mechanisms in environments polluted by heavy metal ions as well as by SO and NO (Tomsett and Thurman 1988). As numerous works have shown, cadmium intoxication strongly depresses glutamate oxaloacetate transaminase (GOT) activity (Benavides et al. 2005 and literature cited herein). Measurement of enzyme capacity of GOT and GDH (glutamate dehydrogenase) is recommended as a useful criterion for the evaluation of the phytotoxicity of soils contaminated by zinc and/or cadmium (Van Assche et al. 1998). Glutamate dehydrogenase also plays an essential role in the adaptation of plants to ammonia assimilation, SO₂ and other toxic gas detoxification (Van Assche et al. 1998). Studies on phosphoglucomutase (PGI) showed its relevance to oxygen availability, soil type and pH, and according to Van Assche et al. (1998), it is likely to be under the control of selection as an enzyme involved in the process of glycolysis. Research conducted on the Scots pine with regard to the above-described allozyme data revealed clear differences between genotypes of trees resistant and susceptible to heavy metals (Chudzińska 2013; Chudzińska et al. 2013a, b).

11.3.2 Mechanisms and Stability of DNA: Heavy Metal Impact

Most of the cellular and molecular aspects of metal toxicity in trees are unknown, even though deleterious effects and the mortality of trees have been recognized.

According to Macnair (Macnair 1993) and Ernst (Ernst 2006), genes for the tolerance of metals are pre-existing at low frequency in non-tolerant populations of certain plant species. In a number of genetic studies, adaptive metal tolerance has been shown to be governed by a small number of major genes with possible contributions from some minor modifier genes (Macnair et al. 2000; Schat et al. 2000). The mechanisms of genetic control and the major genes controlling heavy metal tolerance in trees have not been identified; thus, genetic variation can only be demonstrated indirectly by measuring the responses of different genotypes. The adaptive processes include changes in gene expression patterns, which ultimately lead to biochemical, cellular and physiological changes. To understand the process of plant response to environmental stresses, it is necessary to know the function of crucial genes and phytohormones and their regulation during different phases of the life cycle (Liu et al. 1998; Mukhopadhyay et al. 2004).

Transcriptional factors regulating the stress-responsive gene expression play important roles in stress adaptation (Stockinger et al. 1997; Liu et al. 1998). Tang et al. (2005) found that overexpression of an ERF/AP2 pepper transcription factor (CaPF1) in transgenic *Pinus virginiana* Mill. confers tolerance to heavy metals. Antioxidant enzymes, ascorbate peroxidase (APOX), glutathione reductase (GR) and superoxide dismutase (SOD), demonstrated higher levels of enzyme activity in transgenic Virginia pine plants overexpressing the CaPF1 gene, which may protect cells from the oxidative damage caused by stresses. Kovalchuk et al. (2004) found that the genomic DNA of pine trees exposed to radiation was considerably hyper-methylated. Hyper-methylation appeared to be dependent upon the radiation dose absorbed by the trees and may be viewed as a defence strategy of plants that prevents genome instability and reshuffling of the hereditary material, allowing survival in an extreme environment.

The study of DNA damage and repair is crucial, as DNA is the fundamental unit of inheritance and reproduction, so that perturbations in its structure and function could lead to changes in population dynamics or demography (Theodorakis 2001). The ideal molecular marker for the study of adaptive variations should comply with the following criteria: (1) has an identified DNA sequence, (2) is directly involved in the genetic control of adaptive traits and (3) has easily identifiable allelic variation (González-Martínez et al. 2006). Among the molecular markers that have been used to study populations of metal-tolerant plants are RAPD (random amplified polymorphic DNA), SSR (simple sequence repeats) or ISSR (inter-simple sequence repeat) (Nkongolo et al. 2001; Deng et al. 2007). Due to its hyper-variability, microsatellite DNA (simple sequence repeats, nuSSRs) is often used in studies of coniferous trees (Nowakowska 2006). The application of nuclear microsatellites as codominant markers in plant population studies is related to their ability to define genotypes unambiguously and makes it possible to assess genetic variation of populations growing under strong stress conditions. Microsatellites, found both in coding and non-coding regions in eukaryotic genomes, are markers whose usage is mainly based on the assumption that they are selectively neutral (Ellegrin 2004 and literature cited herein). However, some reports indicate that natural selection can control the level of variability in the

microsatellite loci, although a general function of these markers is not yet known. Chambers and MacAvoy (2000) discuss in detail the potential tasks of microsatellite DNA, such as chromatin organization or regulation of gene activity, demonstrating that microsatellites may be under selection. Observed SSR diversity associated with such different ecological factors as elevated CO₂ level, climate changes or soil contamination suggests that these simple sequence repeats are not necessarily biologically neutral (Rocha et al. 2002; Badri et al. 2008). Mutation rates in nuSSRs are highly compared to mutations at coding gene loci, which makes these markers useful in studying adaptive responses of plants to stress. González-Martínez et al. (2006) worked on the polymorphism pattern of 18 candidate gene responses in *Pinus taeda* for drought stress and identified microsatellites in all those genes. Similar results were obtained by Eveno et al. (2008) in *Pinus pinaster* and by Fluch et al. (2011) in *Picea abies*. In this context, the study of variation in SSRs is a suitable method, but it should be complemented by other molecular techniques, e.g. differential-display reverse transcriptase PCR, in order to identify expressed genes which are involved in various stress responses and in the adaptive process.

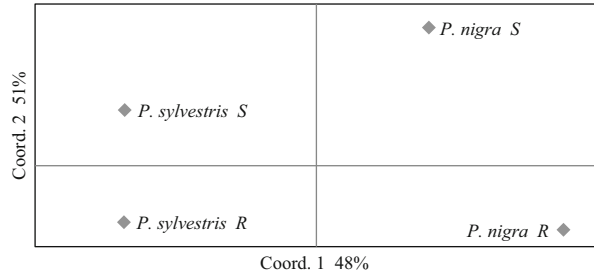
11.3.3 Population Genetics: Heavy Metal Impact

Heavy metal impact on plant genetic structure, besides somatic effects (e.g. DNA damage), may include population genetic effects (changes in genetic diversity or gene frequencies). At the population level, concordant responses between changes in population genetic structure and elevated levels of DNA damage may indicate that the population genetic changes are influenced by exposure to toxic substances. If the frequencies of alleles or other genetic parameters differ between contaminated and reference populations, connections between DNA damage and genotype may provide evidence that these changes are due to toxicant-induced selection (Theodorakis 2001). Environmental contamination may act as a stress-selective factor affecting the genetic structure of the population and result in the extinction of the most vulnerable individuals or, in extreme cases, extinction of entire population. Among the conifer populations exposed to different pollutants, impoverishment of the gene pool is often observed (Geburek et al. 1987; Korshikov et al. 2002). Enzymatic studies of *Picea abies* revealed genetic differences between groups of sensitive trees in polluted areas (Scholz 1991). Observations of higher heterozygosity in tolerant plants of European beech (Müller-Starck 1985), Scots pine and *Populus tremuloides* (Geburek et al. 1987) have been reported.

Another parameter of interest is population diversity. Effects of contaminants on genetic diversity within the population are important because the level of genetic biodiversity affects a population's ability to adapt to natural changes in the environment (Hartl and Clark 1997). Many coniferous species with high genetic diversity are more viable and have better adaptation capacities to unfavourable conditions of an industrially polluted environment (Scholz 1991; Prus-Głowacki and Godzik 1991). This is in accordance to Lerner's classical hypothesis (1954) of

heterozygotic adaptive advantage (as compared to homozygotes), due to the greater capacity of heterozygotes to maintain genetic and physiological homeostasis. Lerner's hypothesis leads to the conclusion that high genetic variability or extensive genetic richness (expressed in the high number of various alleles present in heterozygotic combination) is required for the progress of adaptive processes. Studies on Scots pine populations from natural regeneration and experiments on seedling survival in the vicinity of high levels of heavy metals showed that surviving individuals manifested a high level of homozygosity, particularly evident in some isoenzymatic loci, such as SHDH, MDH, GDH and ADH. A lower number of alleles have also been observed (Prus-Głowacki and Godzik 1991). In studies of *P. sylvestris* growing in areas surrounding operating metallurgical plants, Korshikov et al. (2002) reported a decrease in parameters such as the number of genotypes, alleles per locus and observed and expected heterozygosity. Prus-Głowacki et al. (2006) observed that the presence of heavy metals in soils, combined with SO₂ air pollution, resulted in a potential reduction in genetic variation in *P. sylvestris* populations. This type of adaptive strategy, linked to high level of environmental stress (i.e. heavy metals as pollutants), which drastically narrow the ecological niche, involves selection of the individuals of the specific genetic structure which is suitable in the specific, altered environment. In such cases, high genetic variability no longer presents an adaptive advantage and may be even disadvantageous for the population. This type of adaptation of forest trees to a polluted environment was observed in Norway spruce, European silver fir, common beech and black and Scots pine (Longauer et al. 2001; Korshikov et al. 2002; Schaberg et al. 2008). A decrease in heterozygosity within individuals can be associated with decreased resistance to diseases and might affect population growth. Interesting results are presented in the work of Chudzińska et al. (2013a, b) and Chudzińska (2013) on naturally regenerated trees (35 years old) of *P. sylvestris* and *P. nigra* existing in a highly heavy metal-contaminated site. Sensitive and resistant trees, which differed in fitness, exhibited differences in their genetic structures exhibited in isoenzyme markers and microsatellite loci of nuclear DNA. The greatest differentiation between the two subpopulations was observed in the loci of enzymes involved in metabolic pathways, whose activity is modified by heavy metal ions (Fig. 11.2). Sharma and Dubey (2005) listed over 20 of such enzymes. The Ewens-Watterson test for neutrality and the test of Vitalis et al. (2001) confirmed that allele frequencies at locus GOT B, as well as GDH, MDH C, NDH and SHDH B, of *P. nigra* have been influenced by selection, acting either directly on this locus or on adjacent genomic regions. Among the analysed microsatellite loci of nuclear DNA, two (PtTX 4001 and SPAC 11.4) appeared to be under balancing selection (Chudzińska 2013). SSR markers showed lower or higher genetic diversity of sensitive and resistant subpopulations with respect to certain loci. Two explanations for this phenomenon are most probable: first, those microsatellites lie in close proximity of the gene and are selected because of hitchhiking, and second, microsatellites themselves can be under selection (Meyer et al. 2010). The adaptation strategy of resistant Scots and black pine trees is the result of two processes acting in different loci: (1) strong directional

Fig. 11.2 Principal Coordinates Analysis via Nei's genetic distance (isozyme codominant markers) between sensitive (S) and resistant (R) subpopulations of *P. sylvestris* and *P. nigra* from Miasteczko Śląskie experimental trial in Poland



selection favouring strictly specialized homozygous genotypes (reduction in the level of genetic variation) and (2) stabilizing selection increasing heterozygote frequency (elimination of homozygotes) (Chudzińska et al. 2013a, b).

Both types of adaptive strategy, selection against homozygotes or heterozygotes, result in narrowing of the population gene pool due to elimination of sensitive individuals, which can carry unique alleles. The loss of genetic diversity in populations subjected to anthropogenic stress may be regarded as a factor of concern in risk assessment of heavy metals. This may diminish the genetic variability and fitness of affected populations and make them more susceptible to extinction following a subsequent stress (Guttman 1994).

11.4 Conclusions and Future Research

The heavy metal stress affects plants as a complex and dynamic system of highly variable factors; therefore, plants have many kinds of integrated mechanisms of resistance. It is generally accepted that heavy metals can affect DNA organization, function or gene expression at the individual tree level as well as on the genetic structure at the population level. The occurrence of heavy metal tolerance in plants is connected with the process of selection and microevolution.

Although our knowledge of heavy metal impact on plants has expanded in the last years, mechanisms of tolerance of forest trees are still unclear. To clarify the function of genes of forest trees and knowledge of mechanisms of metal tolerance, further studies are needed. Issues, such as which genes coordinate the improvement of resistance to heavy metal stress, how effectively are contaminated soils able to recultivate or in which way natural forests revive, still remain unanswered. However, the increased use of genetic molecular techniques should have a significant impact on our understanding of heavy metal tolerance and be helpful in the use of plants to remove contaminants from the environment by dendroremediation. This method offers a cost-effective alternative for large areas of unused lands that have been contaminated by heavy metals, especially in the context of tree biotechnology (Nevo et al. 2001). The improvement of the phytoremediation properties of trees can be achieved through modification of their primary and secondary metabolism

(Davison 2005). The example of hybrid poplar, engineered with a modified mercuric ion reductase gene, shows that trees are able to sustain high ionic mercury concentrations (Nevo et al. 2001). Transgenic poplars with higher peroxidase activity showed increased tolerance to zinc due to an enhanced ability to detoxify reactive oxygen species (Bittsanszky et al. 2005). Still, this technology is still in the developmental stage, and the field testing of transgenic plants for phytoremediation is very limited (Hur et al. 2011).

An equally important aim is connected with reintroduction of natural forests on soil contaminated by heavy metals. Mycorrhiza fulfils an important role here. Pine seedlings inoculated with metal-tolerant ecotypes of ectomycorrhizal fungi *Suillus* have lower metal concentrations in their needles than seedlings inoculated with sensitive strains, indicating that the metal-tolerant isolates restrict metal transfer more effectively. The evolutionary adaptation in *Suillus* species contributes to the survival of host trees in metalliferous soils and might be exploited in phytostabilization strategies for heavy metal-contaminated soils (Colpaert et al. 2011). Because of the key role of the ectomycorrhizal symbiosis in tree fitness, the evolution of Cd tolerance in ectomycorrhizal *S. luteus* can be of major importance for the establishment of pine forests on Cd-contaminated soils (Krznaric et al. 2009).

The capacity of plant populations to adapt to the environmental stress is influenced by their genetic potential. Adaptation can only take place if the population possesses or generates appropriate genetic variation. It should be understood that organism adaptation to stress involves genetic costs, namely, gene pool erosion and reduction of genetic variation. Tolerant individuals (genotypes) have to spend a significant part of their life energy to 'the defence' at different levels, which affects their viability and fertility.

After so many years of studies and thousands of well-documented experiments and observations, only one thing is known for sure: in order to describe the effects of pollution effectively, as many aspects as possible should be considered at the same time. For this reason, the value of integrating knowledge of plants in heavy metal-polluted environment should be underlined for making informative management and conservation implications.

References

- Badri M, Zitoun A, Ilahi H, Huguet T, Aouani M (2008) Morphological and microsatellite diversity associated with ecological factors in natural populations of *Medicago laciniata* Mill. (Fabaceae). *J Genet* 87(3):241–255
- Benavides MP, Gallego SM, Tomaro ML (2005) Cadmium toxicity in plants. *Bras J Plant Physiol* 17(1):21–34
- Bergmann F, Hosius B (1996) Effects of heavy-metal polluted soils on the genetic structure of Norway spruce seedlings populations. *Water Air Soil Poll* 89:363–373
- Bittsanszky A, Kömives T, Gullne G, Gyulai G, Kiss J, Heszky L, Radimszky L, Rennenberg H (2005) Ability of transgenic poplars with elevated glutathione content to tolerate zinc (2+) stress. *Environ Int* 31:251–254

- Butorina AK, Kalaev VN, Mazurova SA, Doroshev EV (2001) Cytogenetic variation in populations of Scots pine. *Russ J Ecol* 32(3):198–202
- Butorina AK, Vostrikova TV (2006) Cytogenetic responses of birch to stress factors. *Biol Bull* 33(2):85–190
- Chambers GK, MacAvoy ES (2000) Microsatellites: consensus and controversy. *Comp Biochem Physiol B* 126:455–476
- Chudzińska E (2013) Genetic diversity of Scots pine (*Pinus sylvestris* L.) as an expression of adaptation to heavy industrial pollution: a case study of the population from Miasteczko Śląskie. Wydawnictwo Naukowe UAM, Seria Biologica, Poznań
- Chudzińska E, Diatta J, Póltorak W (2013a) Adaptation strategies and referencing trial of Scots and black pine populations subjected to heavy metal pollution. *Environ Sci Pollut Res* 21:2165–2177. doi:10.1007/s11356-013-2081-3
- Chudzińska E, Pawlaczyk EM, Celiński K, Diatta J (2013b) Response of Scots pine (*Pinus sylvestris* L.) to stress induced by different types of pollutants—testing the fluctuating asymmetry. *Water Environ J* 28:533–539. doi:10.1111/wej.12068
- Chudzińska E, Urbaniak L (2008) *Pinus sylvestris* L. response to heavy metal contamination express in anatomical traits of needles. *Manag Environ Protect Forests* 2:72–84
- Colpaert JV, Wevers JHL, Krznanic E, Adriaensen K (2011) How metal-tolerant ecotypes of ectomycorrhizal fungi protect plants from heavy metal pollution. *Ann Forest Sci* 68:17–24
- Davison J (2005) Risk mitigation of genetically modified bacteria and plants designed for bioremediation. *J Ind Microbiol Biotechnol* 32:639–650
- Deng DM, Shu WS, Zhang J, Zou HL, Ye ZH, Wong MH, Lin Z (2007) Zinc and cadmium accumulation and tolerance in populations of *Sedum alfredii*. *Environ Pollut* 147:381–386
- Derome J, Saarsalmi A (1999) The effect of liming and correction fertilization on heavy metal and macronutrients concentrations in soil solutions in heavy metal polluted Scots pine stands. *Environ Pollut* 104:249–259
- Diatta JB, Chudzinska E, Wirth S (2008) Assessment of heavy metal contamination of soils impacted by a zinc smelter activity. *J Elementol* 13(1):5–16
- Diatta JB, Wirth S, Chudzinska E (2011) Spatial distribution of Zn, Pb, Cd, Cu, and dynamics of bioavailable forms at a Polish metallurgical site. *Fresen Environ Bull* 20(4):976–982
- Dickinson NM, Turner AP, Lepp NW (1991) How do trees and other long lived plants survive in polluted environments. *Funct Ecol* 5:5–11
- Ellegren H (2004) Microsatellites: simple sequences with complex evolution. *Nat Rev Genet* 5:435–445
- Ernst WHO (2006) Evolution of metal tolerance in higher plants. *Forest Snow Landscape Res* 80:251–274
- Eveno E, Collada C, Guevara MA, Léger V, Soto A et al (2008) Contrasting patterns of selection at *Pinus pinaster* Ait. drought stress candidate genes as revealed by genetic differentiation analyses. *Mol Biol Evol* 25(2):417–437
- Freeman JL, Persans MW, Nieman K, Albrecht C, Peer W, Pickering IJ, Salt DE (2004) Increased glutathione biosynthesis plays a role in nickel tolerance in *Thlaspi* nickel hyperaccumulators. *Plant Cell* 16:2176–2191
- Fluch S, Burg A, Kopecky D, Homolka A, Spiess N, Vendramin G (2011) Characterization of variable EST SSR markers for Norway spruce (*Picea abies* L.). *BMC Res Notes* 4:401–412
- Geburek T, Scholz F, Knabe W, Vornweg A (1987) Genetic studies by isoenzyme gene loci on tolerance and sensitivity in an air polluted *Pinus sylvestris* field trial. *Silv Genet* 36:49–53
- Godbold DL (1998) Stress concepts and forest trees. *Chemosphere* 35(4–5):859–864
- González-Martínez SC, Ersoz E, Brown GR, Wheeler NC, Neale DB (2006) DNA sequence variation and selection of tag single nucleotide polymorphisms at candidate genes for drought-stress response in *Pinus taeda* L. *Genetics* 172:1915–1926
- Guttman SI (1994) Population genetic structure and ecotoxicology. *Environ Health Perspect* 102 (Suppl 12):97–100

- Hartl DL, Clark AG (1997) Principles of population genetics. Sinauer Associates, Inc., Sunderland, MA
- Harju L, Saarela KE, Rajander J, Lill JO, Lindroos A, Heselius SJ (2002) Environmental monitoring of trace elements in bark of Scots pine by thick—target PIXE. *Nucl Instrum Methods* 189:163–167
- Hur M, Kim Y, Song H, Kim JM, Choi YI, Yi H (2011) Effect of genetically modified poplars on soil microbial communities during the phytoremediation of waste mine tailings. *Appl Environ Microbiol* 77:7611–7619
- Ivanov YV, Savochkin YV, Kuznietzov V (2011) Scots pine as a model plant for studying the mechanisms of conifers adaptation to heavy metal action: 1. Effects of continuous zinc presence on morphometric and physiological characteristics of developing pine seedlings. *Russ J Plant Physiol* 58(5):871–878
- Kabata-Pendias A (2004) Soil–plant transfer of trace elements: an environmental issue. *Geoderma* 122:143–149
- Kalashnik NA (2008) Chromosome aberration as indicator of technogenic impact of Conifer stands. *Russ J Ecol* 39(4):261–271
- Kim GH, Bell JN, Power SA (2003) Effects of soil cadmium on *Pinus sylvestris* L. seedlings. *Plant Soil* 257:443–449
- Klánová J, Čupr P, Baráková D, Šeda Z, Anděl P, Holoubek I (2009) Can pine needles indicate trends in the air pollution levels at remote sites? *Environ Pollut* 157(12):3248–3254
- Korshikov II, Velikorydko TI, Butil'skaya IA (2002) Genetic structure and variation in *Pinus sylvestris* L. populations degrading due to pollution-induced injury. *Silv Genet* 51(2–3):45–49
- Kovalchuk I, Abramov V, Pogribny I, Kovalchuk O (2004) Molecular aspects of plant adaptation to life in the Chernobyl zone. *Plant Physiol* 135:357–363
- Kozlov MV, Niemelä P, Junttila J (2002) Needle fluctuating asymmetry is a sensitive indicator of pollution impact on Scots pine (*Pinus sylvestris*). *Ecol Indic* 1:271–277
- Kozlov MV, Zvereva E (2011) A second life for old data: global patterns in pollution ecology revealed from published observational studies. *Environ Pollut* 159:1067–1075
- Kozlov MV, Zvereva E, Zverev V (2009) Impacts of point polluters on Terrestrial Biota. *Environ Pollut* 15:197–224
- Kozłowski TT, Pallardy SG (2002) Acclimation and adaptive responses of woody plants to environmental stresses. *Bot Rev* 68:270–334
- Krznicaric E, Verbruggenc N, Wevers JHL, Carleer R, Vangronsveld J, Colpaert JV (2009) Cd-tolerant *Suillus luteus*: a fungal insurance for pines exposed to Cd. *Environ Pollut* 157(5):1581–1588
- Lerner IM (1954) Genetic homeostasis. Olivier and Boyd, Edinburgh
- Lin JX, Sampson DA, Ceulemans R (2001) The effect of crown position and tree age on resin-can density in Scots pine (*Pinus sylvestris* L.) needles. *Can J Bot* 79:1257–1261
- Linhart YB, Grant MC (1996) Evolutionary significance of local genetic differentiation in plants. *Annu Rev Ecol System* 27:237–277
- Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Goda H, Shimada Y, Yoshida S, Shinozaki K, Yamaguchi-Shinozaki K (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. *Plant Cell* 10:391–406
- Longauer R, Gomory D, Paule L, Karnosky F, Mankovska B et al (2001) Selection effects of air pollution on gene pools of Norway spruce, European silver fir and European beech. *Environ Poll* 115:405–411
- Macnair MR (1993) The genetics of metal tolerance in vascular plants. *New Phytol* 124:541–559
- Macnair MR, Tilstone GH, Smith SE (2000) The genetics of metal tolerance and accumulation in higher plants. In: Banuelos G, Terry N (eds) *Phytoremediation of contaminated soil and water*. CRC, Boca Raton, pp 235–250

- Manara A (2012) Plant responses to heavy metals toxicity. In: Furini A (ed) Plants and heavy metals, Briefs in biometals. Springer, New York, pp 27–52
- Markert B, Wünschmann S, Diatta J, Chudzińska E (2012) Innovative observation of the environment: bioindicators and biomonitors: definitions, strategies and applications. Environ Protect Nat Resour 37(2):115–152
- Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic, London
- Mehes-Smith M, Nkongolo K, Cholewa E (2013) Coping mechanisms of plants to metal contaminated soil. In: Dr. Steven Silvern (ed) Environmental change and sustainability. InTech. ISBN:978-953-51-1094-1
- Meyer CL, Kostecka AA, Saumitou-Laprade P, Creach A, Castric V, Pauwels M, Frerot H (2010) Variability of zinc tolerance among and within populations of the pseudometallophyte species *Arabidopsis halleri* and possible role of directional selection. New Phytol 185:130–142
- Mičieta K, Murin G (1998) Tree species of genus *Pinus* suitable as bioindicators of polluted environment. Water Air Soil Poll 104:413–422
- Mukhopadhyay A, Vij S, Tyagi AK (2004) Overexpression of a zinc-finger protein gene from rice confers tolerance to cold, dehydration, and salt stress in transgenic tobacco. Proc Natl Acad Sci U S A 101:6309–6314
- Müller-Starck G (1985) Genetic differences between tolerant and sensitive beeches (*Fagus sylvatica* L.) in an environmentally stressed adult forest stand. Silv Genet 34:241–246
- Nevo E, Beharav A, Meyer MC, Hackett CA, Forster BP, Russell JR, Handley LL, Peña L, Séguin A (2001) Recent advances in the genetic transformations of trees. Trends Biotechnol 19 (12):500–506
- Nieminen T, Helmisaari H-S (1996) Nutrient translocation in the foliage of *Pinus sylvestris* L. growing along a heavy metal pollution gradient. Tree Physiol 16:825–831
- Nkongolo KK, Deck A, Michael P (2001) Molecular and cytological analyses of *Deschampsia cespitosa* populations from Northern Ontario (Canada). Genome 44:818–825
- Novikova TN, Milyutin LI (2006) Variation in certain characters and properties of Scotch pine needles in geographic cultures. Russ J Ecol 37(2):90–96
- Nowakowska J (2006) Zastosowanie markerów DNA (RAPD, SSR, PCR-RFLP i STS) w genetyce drzew leśnych, entomologii, fitopatologii i lowiectwie. Leśne Prace Badawcze 1:73–101
- Oficerov E, Igonina S (2008) Genetic consequences of irradiation in a Scots Pine (*Pinus sylvestris* L.) population. Russ J Genet 45(2):183–188
- Ogawa K, Iwabuchi M (2001) A mechanism for promoting the germination of *Zinnia elegans* seeds by hydrogen peroxide. Plant Cell Physiol 42:286–291
- Oleksyn J, Prus-Głowacki W, Giertych M, Reich PB (1994) Relation between genetic diversity and pollution impact in an experiment with East-European *Pinus sylvestris* provenances. Can J Forest Res 24:2390–2394
- Palowski B (2000) Seed yield from polluted stands of *Pinus sylvestris* L. New Forests 20(1):15–22
- Potters G, Pasternak TP, Guisez Y, Palme K, Jansen M (2007) Stress-induced morphogenic responses: growing out of trouble? Trends Plant Sci 12(3):98–102
- Prasad MNV, Hagemeyer J (2004) Heavy metal stress in plants: from molecules to ecosystems. Springer, Berlin
- Prus-Głowacki W, Chudzińska E, Wojnicka-Półtorak A, Kozacki L, Fagiewicz K (2006) Effects of heavy metal pollution on genetic variation and cytological disturbances in the *Pinus sylvestris* L. population. J Appl Genet 47(2):99–108
- Prus-Głowacki W, Godzik S (1991) Changes induced by zinc smelter pollution in the genetic structure of pine (*Pinus sylvestris* L.) seedling population. Silv Genet 40(5/6):184–188
- Rauser WE (1999) Structure and function of metal chelators produced by plants. The case for organic acids, amino acids, phytin and metallothioneins. Cell Biochem Biophys 31:19–48
- Rice KJ, Emery NC (2003) Managing microevolution: restoration in the face of global change. Front Ecol Environ 1(9):469–478
- Rocha EP, Matic I, Taddei F (2002) Over-representation of repeats in stress response genes: a strategy to increase versatility under stressful conditions? Nucleic Acids Res 30:1886–1894

- Salt DE, Rauser WE (1995) MgATP-dependent transport of phytochelators across the tonoplast of oat roots. *Plant Physiol* 107:1293–1301
- Schaberg PG, DeHayes D, Hawley GJ, Nijensohn S (2008) Anthropogenic alterations of genetic diversity within tree populations: Implications for forest ecosystem resilience. *Forest Ecol Manag* 256:855–862
- Sharma P, Dubey RS (2005) Lead toxicity in plants. *Braz J Plant Physiol* 17:135–152
- Schat H, Llugany M, Bernhard R (2000) Metal-specific patterns of tolerance, uptake and transport of heavy metals in hyperaccumulating and nonhyperaccumulating metallophytes. In: Terry N, Banuelos G (eds) *Phytoremediation of contaminated soils and water*. CRC, Boca Raton, pp 171–188
- Scholz F (1991) Population-level processes and their relevance to the evolution in plants under gaseous air pollutants. In: Taylor GE, Pitelka LF, Clegg MT (eds) *Ecological genetics and air pollution*. Springer-Verlag, New York, pp 167–176
- Schützendübel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *J Exp Bot* 53:1351–1365
- Schützendübel A, Schwanz P, Teichmann T, Gross K, Langenfeld-Heyser R, Godbold DL, Polle A (2001) Cadmium-induced changes in antioxidative systems, hydrogen peroxide content, and differentiation in Scots pine roots. *Plant Physiol* 127:887–898
- Sedelnikova TS, Muratova EN (2001) Karyological study of *Pinus sylvestris* (Pinaceae) with Witches' Broom growing on bog. *Bot Zurnau* 86(12):50–60
- Stockinger EJ, Gilmour SJ, Thomashow MF (1997) *Arabidopsis thaliana* CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. *Proc Natl Acad Sci U S A* 94:1035–1040
- Tang W, Charles TC, Newton RJ (2005) Overexpression of the pepper transcription factor CaPF1 in transgenic Virginia pine (*Pinus virginiana* Mill.) confers multiple stress tolerance and enhances organ growth. *Plant Mol Biol* 59:603–617
- Theodorakis CW (2001) Integration of genotoxic and population genetic endpoints in biomonitoring and risk assessment. *Ecotoxicology* 10:245–256
- Tomsett AB, Thurman DA (1988) Molecular biology of metal tolerances of plants. *Plant Cell Environ* 11:383–394
- Van Assche F, Cardinaels C, Clijsters H (1998) Induction of enzyme capacity in plants as a result of heavy metal toxicity: dose–response relations in *Phaseolus vulgaris* L., treated with zinc and cadmium. *Environ Pollut* 52(2):103–115
- Van Straalen N, Timmermans MJTN (2002) Genetic variation in toxicant-stressed populations: an evaluation of the “genetic erosion” hypothesis. *Hum Ecol Risk Assess* 8(5):983–1002
- Vitalis R, Dawson K, Boursot P (2001) Interpretation of variation across marker loci as evidence of selection. *Genetics* 158:1811–1823
- Waalkes MP, Goering PL (1990) Metallothionein and other cadmium-binding proteins: recent developments. *Chem Res Toxicol* 3:281–288
- Xue TT, Li XZ, Zhu W, Wu CA, Yang GD, Zheng CC (2009) Cotton metallothionein *GhMT3a*, a reactive oxygen species scavenger increased against abiotic stress in transgenic tobacco and yeast. *J Exp Bot* 60:339–349
- Yadav SK (2010) Heavy metals toxicity in plants: an overview on the role of glutathione and phytochelators in heavy metal stress tolerance of plants. *S Afr J Bot* 76:167–179