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*Review*

# Macro and Micronutrient Storage in Plants and Their Remobilization When Facing Scarcity: The Case of Drought

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**Abstract:** Human mineral malnutrition or hidden hunger is considered a global challenge, affecting a large proportion of the world's population. The reduction in the mineral content of edible plant products is frequently found in cultivars bred for higher yields, and is probably increased by intensive agricultural practices. The filling of grain with macro and micronutrients is partly the result of a direct allocation from root uptake and remobilization from vegetative tissues. The aim of this bibliographic review is to focus on recent knowledge obtained from ionomic analysis of plant tissues in order to build a global appraisal of the potential remobilization of all macro and micronutrients, and especially those from leaves. Nitrogen is always remobilized from leaves of all plant species, although with different efficiencies, while nutrients such as K, S, P, Mg, Cu, Mo, Fe and Zn can be mobilized to a certain extent when plants are facing deficiencies. On the opposite, there is few evidence for leaf mobilization of Ca, Mn, Ni and B. Mechanisms related to the remobilization process (remobilization of mineral forms from vacuolar and organic compounds associated with senescence, respectively) are also discussed in the context of drought, an abiotic stress that is thought to increase and known to modulate the ionic composition of grain in crops.

**Keywords:** grain filling; nutrient remobilization; senescence; abiotic stress

## 1. Introduction

Mineral malnutrition is considered to be the most serious global challenge to humankind with over 60, 30, 30 and 15% of the world population being Fe, Zn, I or Se deficient, respectively. In addition, Ca, Mg and Cu deficiencies are common in many developed and developing countries (see the extensive review [1]). Because plants are the basis of nearly all food chains, the production of biofortified seeds, fruits or edible vegetative organs with increased concentrations of micronutrients could reduce what is considered "hidden hunger".

Plants are sessile organisms that have to cope with a permanently fluctuating environment, both in space and time, and this includes changes in abiotic factors such as light, temperature, water and

nutrient availabilities that are tightly linked to biotic interactions with other plants or rhizosphere microorganisms such as bacteria and fungi. To cope with these variable environments, plants through their evolution have developed multiple mechanisms allowing their survival, growth and reproductive success. For example, the uptake of soil minerals is achieved via many processes to minimize transient deficiency [2–5] including stimulation of root transporter activities, oriented root growth and/or root exudation. Plants may also increase soil mineral availability and improve their nutrient uptake through interactions with rhizospheric microorganisms [6].

Hence, a better understanding of the mechanisms involved in plant nutrient acquisition and distribution in edible products with increased micronutrient concentrations could pave the way to the development of improved plant varieties, and participate in an amelioration of human malnutrition. Seed micronutrient enrichment depends upon the uptake, storage, mobilization and translocation of the micronutrients. All of these complex processes require the coordinated regulation of many genes. For example, a recent study has shown that two chromosome regions were associated with Quantitative Trait Loci for improvement of zinc and iron content in grains [7]. Therefore, breeding for bio-fortification combined with improvement of a well-synchronized supply of various amounts and forms of fertilizers in crop rotations are considered appropriate to alleviate mineral malnutrition [8]. The study by Fan et al. [9] shows that breeding wheat for a better yield could be achieved via enhanced photosynthesis combined with an ample supply of macro-nutrients (N, P, K), but this led to a negative selection for micronutrient content in seeds. These authors analysed mineral concentrations of archived wheat grains and soil samples between 1843 and 2008 in relation to cultivar, yield and harvest index, and reported that the seed micronutrient contents remained stable between 1843 and the mid 1960s but decreased significantly after that time. This coincides with the introduction of semi-dwarf and high-yielding cultivars, while at the same time the soil concentrations of Zn, Fe, Cu and Mg either increased or remained stable. Overall, this indicates that the reduced mineral content of seeds partly resulted from this negative genetic selection and thus manifested as hidden hunger [9].

Additionally, in the context of global changes resulting from increased accumulation of greenhouse gases [10] associated with an increase in temperature and the occurrence of greater risk of aridity [11,12], the mineral contents of seeds may also be adversely affected. For example, a meta-analysis of the literature that considered the effect of increased atmospheric $CO_2$ on the plant ionome [10] indicated in the majority of cases that leaf and seed N content and most of their mineral nutrients (P, K, Ca, S, Mg, Fe, Zn, Cu, Mn) were negatively impacted as the seed carbon concentration increased. This empirically robust relationship was systemic i.e., independent of plant species, cropping areas and experimental designs, including Free-air $CO_2$ enrichment (FACE) experiments. Another meta-analysis [11] revealed that drought stress in plants had a short-term (<90 days) negative effect on N and P contents in plants, alleviated either in the long term or by drying-rewetting cycles.

Soil mineral nutrient deficiency may be the consequence of different constraints including intrinsic low soil mineral contents, sub-optimal abiotic conditions like extreme temperatures, pH, low soil water content or anaerobic conditions that will alter mineralization and hence phytoavailability. When nutrient availability is too low, several strategies have been described that enable plants to cope with most macronutrient and some micronutrient deficiencies, and they rely on (i) an over expression of genes encoding for increased or decreased nutrient specific root transporters coupled in the longer term with (ii) an increased exploration of soil due to enhanced root branching and growth [13,14]. Mineral nutrient deficiencies usually elicit mechanisms that rely mostly on an up-regulation of specific root membrane high- or low-affinity transport systems. These mechanisms have been extensively described for N, P, and S [2,3] and the complexity of their regulation is being progressively unravelled. The involvement of multiple transporters in several regulation pathways suggests that higher plants have a sophisticated uptake system finely tuned to external nutrient availability and internal plant needs. However, although up-regulation of nutrient uptake by roots coupled with increased root exploration and/or microbial stimulation via root exudation are among the common coping strategies,

they can be unsuccessful under certain circumstances. This may be the case when soil nutrient availability remains too low or when low soil water content restricts nutrient mobility.

Besides its implication in photosynthesis and loss of water through the stomatal aperture [15], plant transpiration conveys many functions such as cooling leaves and driving both the mass flow of nutrients from the soil to the rhizosphere and the xylem flow from roots to the shoot. An increase in transpiratory flux is usually correlated with [15] an increase in photosynthesis activity, leading to loss of water when stomata are open for $CO_2$ uptake. Mass flow, driven by transpiration, leads to substantial nutrient delivery to the root surface. For example, plants of *Zea mays* grown in a fertile loam had the potential to accumulate 95% of the S, 88% of the Mg, 79% of the N, 73% of the Ca, 18% the K, and the 5% of P by mass flow, the remainder being taken up through diffusion and uptake by the roots [16]. Similar situations where mass flow and hence nutrient mobility in the soil may be reduced can be found when plant transpiration is low. This may occur during the spring bud growth of deciduous trees, regrowth of forage species subjected to mechanical or animal defoliation, or the spring resumption of growth of hemicryptophyte and cryptophyte plant species. Moreover, this could also be the case during the development of annual species. For example, in *Brassica napus* it has been shown that nutrient uptake (especially N) is considerably reduced during the reproductive stage. Consequently, development of reproductive tissues and seed filling relies on monocarpic senescence, which allows degradation of organic compounds associated with internal recycling of nutrients [17].

Whatever the reasons for decreased nutrient uptake (environmental or developmental), short-term use of macro and micronutrients previously stored within tissues may be an effective alternative for sustaining plant growth. Moreover, in the long term, i.e., when nutrient deficiency becomes severe, degradation of organic compounds (which may also contain micronutrients) is associated with leaf senescence [18,19] in order to provide nutrients useful to sustain plant growth. In such situations, plant growth mostly relies on internal stores than can be mobilized from the short term to the long term. However, the importance of nutrient storage and recycling are not always recognized. Thus, some authors [3] consider that recycling of pools of essential macronutrients (N, P, S) previously stored in plant tissues is not sufficient to support normal growth for more than a few days in most higher plants. In contrast, other reports have [19,20] clearly stated that manipulating nutrient remobilization is a major challenge for modern agriculture. For example, some authors suggest that an improvement in nutrient remobilization from senescing tissues constitutes an appropriate lever to increase global nutrient use efficiency (especially N) [17,21] or to biofortify edible products [22–24]. In any case, efficient nutrient mobilization in plants needs to satisfy several conditions, such as the possibility to accumulate large amounts of nutrients in plant parts without reaching a toxicity level, an ability to be moved from storage compartments (e.g., the vacuole) and their capability to be transported [23] between tissues through xylem or phloem vessels (Figure 1). A fourth condition may be a requirement when nutrients have been stored within fairly complex compounds that are either not easily transported by the phloem or the xylem or that require dismantling into mobile and nontoxic forms.

The overall objectives of this review are to focus on the body of knowledge related to plant nutrient mobilization from leaves (the main source organs) to the seeds (the main sink organs) that to our knowledge has never been reviewed at the level of ionome. Moreover, because one of the main effect of drought is to reduce mass flow and hence mineral nutrient uptake, it can be hypothesized that under this abiotic stress, grain filling will mostly rely on leaf nutrient remobilization. In the most part, this review references studies of plant ionome analysis to (i) identify general patterns of leaf accumulation and remobilization according to the nutrient considered and the plant species, (ii) relate these patterns to potential mechanisms (including mineral vacuolar sequestration/mobilization capacity and mobilization of organic compounds associated with senescence), (iii) evaluate from the literature how these mechanisms and the resulting nutrient remobilizations are affected by drought, and finally (iv) estimate from the literature how seed nutrient contents are affected by drought and whether they can be related to leaf remobilization processes.

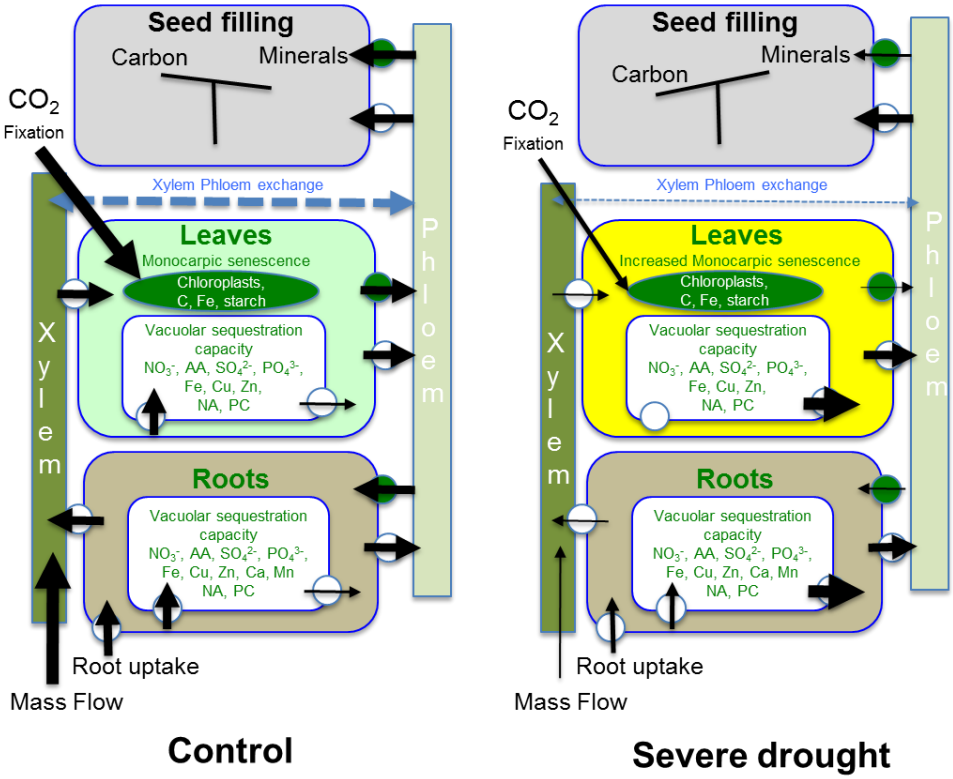



**Figure 1.** Proposed model for leaf remobilization of macro and micronutrients towards seed filling under normal conditions or during severe drought illustrating the involvement of vacuolar sequestration capacity, coupled under severe drought with increased monocarpic senescence and reduced photosynthesis. NA: nicotianamide, PC: phytochelatin.

## 2. General Patterns of Nutrient Mobilization from Leaves

Because of the concomitant root uptake, the quantification of nutrient mobilization from vegetative to reproductive tissues usually requires the use of either stable or radioactive isotopes [25]. Numerous studies have used pulse chase labelling of $^{15}N$ or $^{13}C$, and more recently $^{34}S$ or $^{65}Zn$ [26,27], and very occasionally $^{54}Mn$ [28], but only a few other elements have been considered under very specific circumstances (effects of genetic modifications or environmental constraints such as deficiencies or water supply). Alternatively, changes in the ionomic composition of leaves have been used to evaluate (by mass balance) the net remobilization of most leaf nutrients during irreversible processes such as monocarpic senescence [29–33].

Data from a meta-analysis designed to evaluate specific and genetic variations under a wide range of plant culture conditions, tissues, species and genotypes [1], showed that the ratio between the maximum and minimum contents of nutrients such as Fe, Zn, Mg and Cu have a much wider variation in leaves than in seeds, suggesting a remobilization potential in vegetative tissues. The general pattern of nutrient accumulation and remobilization during leaf development is probably nutrient and species dependent (Table 1), being affected by either nutrient availability or plant phenology (reproductive versus vegetative growth). However, according to the previously cited literature, three types of potential recycling can be hypothesized (Figure 2), at least for nutrients for which enough information is available (Table 1). Irrespective of the plant species, N is always remobilized from leaves (Table 1) and requires a proteolytic system to degrade proteins during leaf senescence. However, depending on the plant species, the efficiency of N mobilization is variable, reaching about 90% in *Triticum aestivum*, *Hordeum vulgare* and *Arabidopsis thaliana* against only 39% in *Zea mays*, while *Brassica napus*, *Pisum sativum* and *Glycine max* show intermediate values (Table 1). Other macronutrients such as K,

S, and P are also remobilized from leaves at a rate similar or lower to N, except in *Z. mays* leaves, which only remobilize K with a fairly high efficiency. Magnesium (Mg) is also remobilized from leaves, except in *Z. mays*, *A. thaliana* and *P. sativum*. Calcium (Ca), is a macronutrient that is considered to have a limited mobility in the phloem. Consequently, Ca stored in leaves is not remobilized, at least not in non-poaceae species. Overall, it should be pointed out that for a given species the rates of mobilization of macronutrients (except for Ca) is relative to that of N withdrawal from leaves (Table 1), suggesting that they depend on leaf senescence. The situation for micronutrients (Table 1) is far more complex, showing more interspecies variations. Boron (except in *Z. mays*) and nickel (except in *B. napus*) seem to be fairly immobile in the leaves of all species, while in contrast copper (Cu) is exported from leaves of all species (Table 1). Manganese (Mn), like Ca as previously reported, is only remobilized in *T. aestivum*, *O. vulgare* and *O. sativa*, which may be the result of a rapid release of water during plant dehydration provoking Mn or Ca movement in the xylem [34,35]. Molybdenum (Mo), iron (Fe) and zinc (Zn) show the greatest variation between species, with *B. napus* and *Z. mays* being unable to export Zn and Mo from the leaves, while Fe is not remobilized in poaceae plants (except at a low level in rice) or in *P. sativum*. Similarly, studies in soybean [36] and wheat [37] revealed that levels of Cu, Fe and Zn declined in leaves undergoing senescence. This apparent heterogeneity in the potential remobilization of micronutrients might be linked to the fact that the requirements for these elements in seeds are fairly low, and might be fulfilled by the translocation of nutrient taken up by the roots post anthesis. Accordingly, Sankaran and Grusak [33] suggested that the remobilization of micronutrients would be required only when soil deficiencies limit root uptake.

Based on the previous information, the patterns of leaf nutrient remobilization could be separated into three nutrient groups (Figure 2). Group I corresponds mostly to N, a highly mobile nutrient with a predominantly organic form of storage (especially proteins), and it is accumulated in leaves up to the beginning of leaf senescence (Figure 2). Subsequently N is exported to young developing leaves and/or reproductive tissues in nearly all plant species. When facing N deficiencies during early or late leaf development, the mobilization rates can usually be increased concomitantly with senescence induction [38–41]. Group II (Figure 2) corresponds to nutrients like K, S, P, Mg, Cu, Fe, Zn and Mo, which are more variable in their mobility and mostly found in either inorganic forms or associated with cellular organic molecules such as proteins that are constantly accumulated during leaf development until full expansion. Their remobilization may occur during monocarpic senescence, but can be increased (probably from mineral storage forms) when plants are facing a deficiency, without necessarily inducing senescence. For example, it has been well characterized in *B. napus* that a moderate S deficiency increases leaf S mobilization (mostly from $SO_4^{2-}$), whereas leaf senescence is delayed [42]. Likewise, in the case of Cu and Fe in wheat [42] and Fe [31] or P [43] in rice, increased remobilization from leaves is triggered by reduced root uptake, which in turn is a consequence of soil mineral deficiencies. Group III (Figure 2) corresponds to fairly immobile nutrients such as Ca, Mn (at least in non poaceae species), B and Ni, which accumulate during the entire leaf life span or reach a plateau after the onset of senescence, without any effect of deficiency on mobilization and/or senescence [32]. Such a lack of remobilization could result from (i) a very low phloem mobility (Ca and Mn), (ii) nutrient sequestration into a fairly complex molecule that is not dismantled during senescence (for example, B and rhamnogalacturonan located in cell walls [44]), or (iii) by potential nutrient toxicity at the cellular level (such as Ni, [45]) precluding its remobilization. In the meantime, whichever nutrient group is considered, the accumulation of all nutrients in leaves can be reduced in a similar way if deficiencies occur in early leaf development (Figure 2) and lead to reduced storage in leaves before seed development. However, while these general patterns of nutrient remobilization from leaves offer an easy way to compare nutrients and plants species, they do not take account of the mechanisms involved. Indeed, processes associated with the mobilization of short- or long-term nutrient storage pools may be affected by the severity of environmental stresses such as drought.

**Table 1.** Nutrient remobilization from senescent leaves in 8 plant species from studies using multi-element analysis. References are identified by superscript numbers. Percentages indicate the relative decrease in mineral content in senescent leaves during grain filling.

| Species | Leaf Remobilization of Macronutrients | | | | | | Leaf Remobilization of Micronutrients | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **N** | **K** | **S** | **P** | **Mg** | **Ca** | **Mn** | **Cu** | **Mo** | **B** | **Ni** | **Fe** | **Zn** |
| *Triticum aestivum* | 93% [1] | 95% [1] | 94% [1] | 89% [1] | 89% [1] | 89% [1] | 43% [1] | 71% [1] | 79% [1] | 0 [1] | 0 [1] | 0 [1] | 92% [1] |
| *Hordeum vulgare* | 88% [1] | 96% [1] | 93% [1] | 87% [1] | 96% [1] | 89% [1] | 70% [1] | 84% [1] | 0 [1] | 0 [1] | 0 [1] | 0 [1] | 70% [1] |
| *Oryza sativa* | - | 47% [4] | 20% [4] | - | 45% [4] | 33% [4] | 65% [4] | 40% [4] | 46% [4] | - | 0 [4] | 20% [4] | 29% [4] |
| *Zea mays* | 39% [1] | 72% [1] | 0 [1] | 0 [1] | 0 [1] | 0 [1] | 0 [1] | 0 [1] | 0 [1] | 59% [1] | 0 [1] | 0 [1] | 0 [1] |
| *Brassica napus* | 51% [1] | 37% [1] | 54% [1] | 55% [1] | 36% [1] | 0 [1] | 0 [1] | 50% [1] | 0 [1] | 0 [1] | 91% [1] | 68% [1] | 0 [1] |
| *Arabidopsis thaliana* | 88% [2] | 86% [2] | 65% [2] | 78% [2] | 0 [2] | 0 [2] | 0 [2] | 54% [2] | 78% [2] | - | 0 [2] | 55% [2] | 54% [2] |
| *Glycine max* | 60% [5] | 63% [5] | - | 67% [5] | 49% [5] | 59% [5] | 42% [5] | 60% [5] | 96% [5] | 45% [5] | - | 38% [5] | 48% [5] |
| *Pisum sativum* | 72% [1] | 43% [1] | 57% [1] | 81% [1] | 0 [1] | 0 [1] | 0 [1] | 0 [1] | 0 [1] | 0 [1] | 0 [1] | 0 [1] | 47% [1] |
| | - | 50% [3] | 65% [3] | 82% [3] | 50% [3] | - | 21% [3] | 73% [3] | - | - | - | 70% [3] | 32% [3] |

References: [1] [32]; [2] [30]; [3] [33]; [4] [31]; [5] [36].

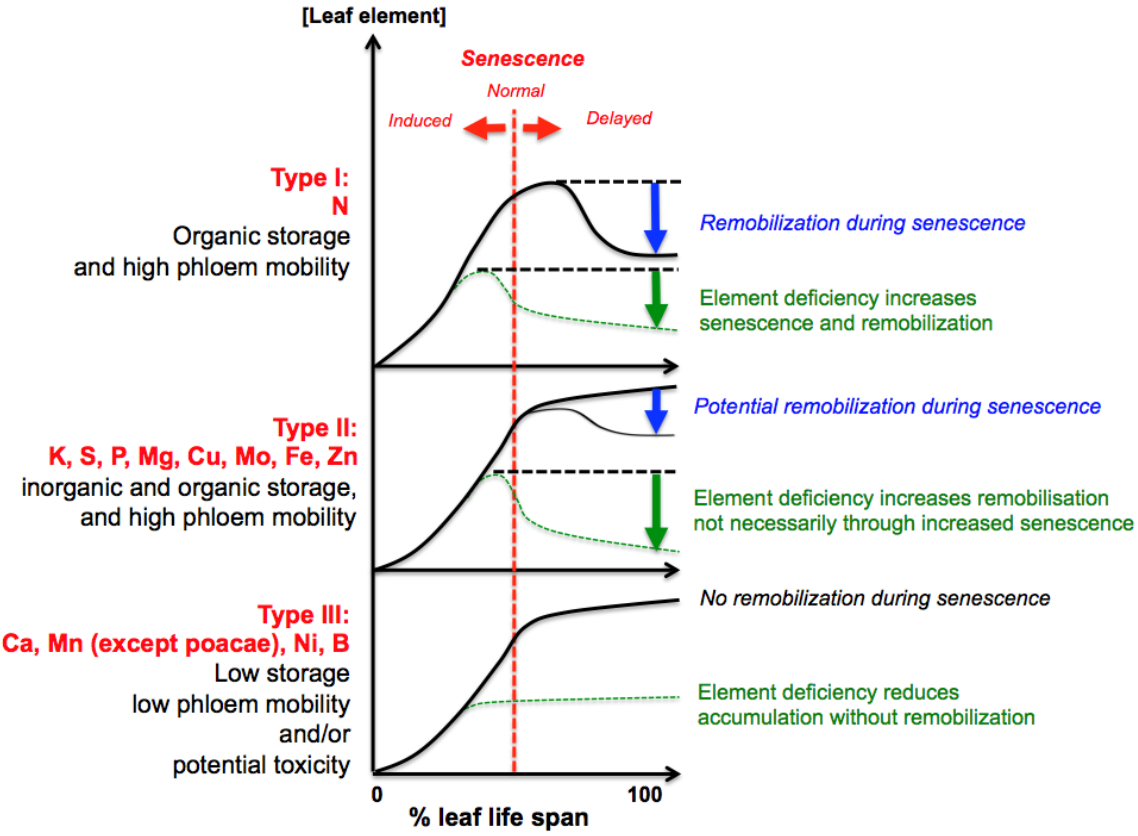



**Figure 2.** General patterns of macro and micronutrient contents of leaves resulting from their allocation from root uptake before senescence followed by their remobilisation (net loss) as a function of relative leaf lifespan. According to the macro or micronutrient considered, senescence and/or nutrient deficiency induce high (Type I), variable (Type II) or low remobilization (Type III) from the leaves to the seeds.

## 3. Short-Term Mobilization of Stored Compounds When Facing Nutrient Deficiencies or High Nutrient Needs

For most nutrients, short-term storage compounds are mostly found in the vacuoles (Figure 1), under inorganic ($NO_3^-$, $SO_4^{2-}$, $K^+$, $Mg^{2+}$, PP, Pi) or complexed forms with organic compounds such as organic acids (Ca, Mo), amino acids (Ni, Se), nicotinamide (NA), specific chelates such as ferritin (Fe, although mostly in chloroplasts), and even proteins (Cu and Zn). Mineral micronutrients are thought to be in chelated or ligand-bound forms when not incorporated into proteins, including during their phloem transport (Figure 1). As reported in the review of Peng and Gong [46], studies on long distance transport of nutrients have focused on transporters expressed in xylem parenchyma cells or in phloem companion cells, as well as the role of phytochelators such as NA and phytochelatins. Considering the vacuole as a pivotal organelle for storage of metabolites and minerals, Peng and Gong [46] proposed the concept of vacuolar sequestration capacity (VSC) acting as a buffering pool not only controlling accumulation in cells but also dynamically mediating transport of nutrients over long distances. Complex regulation of the VSC of metals would involve the modulation of (i) the expression of tonoplast specific transporters (MTP1/MTP3/ZIF1 for Zn, NRAMP3/NRAMP4 for Fe, COPT5 for Cu) or other transporters with lower specificity (VIT1/VIT2 for Fe and Zn, FPN2 for Fe, Co and Ni) and (ii) the synthesis of chelators such as NA that modulate the VSC of Fe and Zn [47] and phytochelatin modulating the VSC of Cu. NA, which is also a precursor of phytosiderophores in grasses, has the capacity to bind Cu, Co, Fe(II) and Fe(III), Mn, Ni, and Zn. This body of evidence strongly suggests that NA is a vital chelator of micronutrients for homeostasis during growth, for translocation within

vegetative parts of the plant and also in phloem transport of micronutrients to the seeds. Proteins that transport these NA complexes are the yellow-stripe-like (YSL) proteins, which are members of the oligopeptide transport (OPT) family [48].

While some nutrients such as Ca or Mn are recognized as showing little mobility between tissues, and for which, like Cu and Ni, the transport is poorly understood, others like N, S, P, Fe, Zn are considered to be potentially highly mobile in either xylem or phloem vessels (Figure 1). For example, S is usually recognized as showing variable mobility [49]. In fact, the S content itself in tissues is highly variable and can be correlated with $SO_4^{2-}$ content, which may account for more than 80% of total S in leaves of field grown B. napus [50]. As a consequence, when facing S deficiency, plant growth can be sustained for several weeks [51], provided that enough $SO_4^{2-}$ was accumulated, and thanks to its remobilization and high mobility. In contrast, plants containing only organic S pools (i.e., low mobile compounds) are unable to grow without a significant S supply. A much wider variation was observed for S and $SO_4^{2-}$ concentrations than for N or $NO_3^-$ concentrations, which suggests a smaller contribution of vacuolar $NO_3^-$ to N remobilization, and most of it derived from organic N. However, recent results [52] obtained in B. napus suggest that a decrease in the VSC of $NO_3^-$ in roots enhances N transport to shoots and essentially contributes to higher N use efficiency. This occurs by promoting $NO_3^-$ allocation to aerial parts, likely through coordinated regulation of the $NO_3^-$ tonoplastic transporters, NRT1.5 and NRT1.8. Based on this evidence, there is obviously a relationship between the variable capacity to accumulate a nutrient in vegetative tissue and the potential to remobilize a portion of it when facing a restriction.

## 4. Monocarpic Senescence and Remobilization of Nutrients

Long-term storage may also occur for some nutrients, mostly in a complex form requiring specific catabolic transformations, first to enabling their remobilization, then their transport. This is the case for nutrients that are components of various polymer structures (e.g., N and S in proteins, S and P in lipids, B in rhamnogalacturonan II, Zn in numerous proteins) or involved in their physiological function (Fe and ferritin, Mo as a co-factor, Mg in chlorophyll, Mn as a catalytically active metal in proteins). It is usually assumed that their mobilization is an intrinsic feature of plant senescence [18]. The complex mobilization process of these nutrients, eventually leading to the death of vegetative organs, involves a well-orchestrated activation of genes encoding catabolic enzymes that gradually dismantle cellular components that are mostly located in chloroplasts. At the same time, basic metabolic activities are kept intact until cell death to ensure the processing of high molecular weight components and the subsequent export of the degradation products and minerals to the phloem [53]. Recent research [17,54] suggests that the link between growth and the ageing process is nutritional in nature, by which resources (most of the time, only N is considered) are recycled from obsolete body parts to newly developing structures. For example, feeding the plant with N fertilizer increases the amount of Rubisco but does not change its turnover, and this N responsive behaviour is characteristic of a storage protein. When the N demand cannot be met by uptake from the rhizosphere alone, N, and potentially its associated nutrients, is withdrawn from older tissues. Similarly, it was suggested that sequential senescence (i.e., acropetal senescence occurring during the vegetative stage) is a useful adaptation because it buffers growth against fluctuation in the supply of N and other elements. Results from studies on legumes demonstrate that the N being remobilized from vegetative parts to fill seeds arises from a common N pool translocated throughout the plant [55]. Although various proteomic and metabolomic studies are being conducted, the detailed catabolic pathways involved are not yet fully characterized. The mechanism of autophagy, consisting of the allocation of unnecessary or damaged cytosolic components (such as organelles and macromolecules) for degradation and recycling by the vacuole (see [56] for review), has been recently characterized during senescence [57]. Such a mechanism might explain how nutrients other than N may also be mobilized, as numerous proteins contain elements such as Zn, Cu, Mn, Fe or N.

The results of Distefeld et al. [58], who compared a wild tetraploid wheat (*Triticum turgidum* ssp. *dicoccoides*) to a cultivated wheat (*Triticum durum*), strengthen the hypothesis that senescence process may increase the mobilization of micronutrients other than N. These authors found a higher mobilization of Zn, Mn and Fe in wild wheat and attributed it in part to allelic variation at a chromosome locus that promotes early senescence and remobilization of proteins from senescing leaves to seeds. Waters and Sankaran [23] considered that because Cu, Zn and Fe transport frequently involves chelation with amino acids, remobilization of these elements might be tightly linked with N catabolism occurring during senescence. This could be further supported by the fact that wheat lines with delayed leaf senescence have lower amounts of Fe and Zn in their seeds [59]. Ricachenevsky et al. [48] suggest a major role of some NAC (acronym derived from three genes that were initially discovered to contain a particular domain (the NAC domain): NAM for no apical meristem, ATAF for *Arabidopsis thaliana* activating factor, and CUC2 for cup-shaped cotyledon) transcription factors in wheat and rice that could regulate the onset of leaf senescence, together with an increased Fe and Zn remobilization, through an increased synthesis of nicotianamine associated with a higher expression of a metal-NA transporter (YSL2). Similarly, Pearce et al. [60] showed that a wheat mutant under-expressing *GPC1*, a transcription factor that reduces leaf senescence, had lower Zn and Fe in the seeds, probably as a result of a down regulation of *Zip* and *YSL* genes coding for Fe and Zn transporters. In legumes, leaf and nodule senescence is a programmed process concomitant with the transition to the reproductive stage in the host plant life cycle [55,61]. Van de Verlde et al. [62] highlighted that the transcriptome of nodule senescence shared a high functional overlap with the transcriptome of leaf senescence, indicating a shift from a carbon sink to a nutrient source tissue. In particular, in soybean, it has been estimated that around 50% of the nodule Fe content was recycled to the seeds [63] and in *Lotus japonicus*, a nodule-specific NA synthase has been identified [64]. Taken together, these results suggest that nodules could also contribute to the mobilization of micronutrients to the seeds [65].

## 5. How Drought Affects Leaf Accumulation of Nutrients, Leaf Senescence, Mobilization of Nutrients and Seed Filling

Drought reduces the diffusion rate of nutrients in the soil towards the roots, the nutrient uptake by the roots, and their transport to the shoots due to concomitant decreases in transpiration flux, active transport and membrane permeability (see the review from Hu and Schmidhalter [66]. Drought during the vegetative stage in *Arabidopsis thaliana* induces a decrease in the concentration of almost all minerals including Zn, Fe, Mn, Ca and Mg in leaves, except for K [67]. The authors explain these reductions by a preferential allocation of resources to the roots, at the expense of leaf growth. Otherwise, the increase in leaf K content under drought could be related to its role in maintaining plant water potential and in alleviating drought-induced oxidative damage through inhibition of the production of reactive oxygen species (ROS) [68]. Yet from a mechanistic point of view, how vacuolar storage and long distance transport of nutrients is affected by drought during the reproductive stage is fairly unknown. However, it could be postulated that a reduction in mass flow resulting from drought, and hence a reduction in the phytoavailability of soil nutrients, could be similar to a situation of nutrient deficiency (previously described in paragraph 3). Under this assumption, long distance transport would be regulated through modulation of VSC by the changes in both transporter expression and chelate synthesis (Figure 1). For example, in *Medicago truncatula*, genes coding for sulfate transporters involved in sulfate uptake (*SULTR* of Group 1) are down-regulated in roots, while genes coding for sulfate transporters involved in the efflux of sulfate from the vacuole lumen to the cytosol (*SULTR* of Group 4) are up-regulated in shoots [69]. Consequently, short-term vacuolar storage of nutrients would be of prime importance to buffer transient and moderate drought, which could be, at least partly, independent of senescence.

Among environmental stresses, drought is known to induce leaf senescence, especially via changes in hormonal balance related to the concomitant decrease and increase in cytokinin (CKs) and

abscisic acid (ABA) contents, respectively [70,71]. Drought-induced senescence is associated with numerous morphological, physiological and molecular modifications in several species including nutrient remobilization from senescing organs (mainly leaves, and to a much smaller extent nodules in legumes) to young tissues (leaves or seeds), thus compensating for the nutrient uptake deficit that results from low soil water content [72,73]. While much work has been conducted to evaluate the consequences of drought on seed nutrient contents, to our knowledge studies focusing on the effect of drought specifically on nutrient remobilization from leaves (estimated from a net balance during senescence) are very scarce.

According to Wang and Frei [74], accelerated senescence under many types of stress, including drought, affects nutrient translocation processes, inducing the remobilization of N from vegetative to reproductive plant parts, and shortening the maturation time, which tends to favour proteins over starch accumulation in cereal grains. Decreased mineral content in seeds can therefore result from reduced root uptake and translocation and/or insufficient remobilization from leaves. This contrasts with reports of increases in some mineral contents in seeds following drought, which are probably a result of reduced photosynthesis leading to reduced C content in the seeds. However, most conclusions appear to be heterogeneous if not opposite (Table 2), even within the same plant species. New experimental design, in which both the severity of drought and the availability of nutrients are finely monitored will be required to obtain more relevant data with different plant species. As pointed out in the reviews of Lawlor [75] and He and Dijkstra [11], many different experimental procedures have been used to subject plants to drought, ranging from field to controlled conditions, using PEG (Polyethylen Glycol) or mannitol in nutrient solution, manipulating soil water content from progressive reductions to complete cessation of watering and finally using cycles of soil drying/rewatering.

**Table 2.** Effect of drought on the nutrient contents of seeds in different plant species submitted to severe (% decrease in seed yield is given when available) or moderate drought from studies using multi-element analysis. Drought is considered to be severe as opposed to moderate when it significantly reduced grain yield.

| Species | Drought | Increased Seed Nutrient Content | Decreased Seed Nutrient Content | Refs. |
|---|---|---|---|---|
| *Hordeum vulgare* | Severe | N | P, K, Mg | [76] |
| | Moderate | No effect | No effect | [76] |
| | Severe | N, Zn, Mn | No effect | [77] |
| *Triticum turgidum* | Severe | N, Fe, Zn | No effect | [78] |
| | Moderate | No effect | No effect | [78] |
| *Triticum aestivum* | Severe | N | Not quantified | [79] |
| | Severe | (N not quantified), K, Ca | No effect | [80] |
| | Moderate | | | [80] |
| | Moderate | P, Mg, Zn | K | [81] |
| | Severe | P, Ca, Mg, Zn | K | [81] |
| *Zea mays* | Severe | N, Ca, Mg, Cu, N | P, K | [82] |
| | Severe | N | Fe, Zn, Cu | [83] |
| | Moderate | | Fe, Zn, Cu | [83] |
| *Glycine max* | Moderate | No effect | N, K, P, Ca, Fe | [84] |
| | Severe | P, Ca, Mn, Zn, Mo | No effect | [85] |
| | Severe | Ca, Fe | K, P, Mn, Cu, Zn | [86] |
| | Moderate | Ca, K | P, Mn, Cu, Zn | [86] |
| *Oryza sativa* | Severe | N | Cu, Fe | [87] |
| *Phaseolus vulgaris* | Moderate | | Fe, Zn | [88] |

For example, it has been shown that high molecular weight PEG can be taken up by roots and accumulated in extracellular spaces inducing cellular but not tissue dehydration [89]. Moreover, Verslues et al. [90] showed that the use of PEG in the nutrient solution also decreased dioxygen

movement by increasing solution viscosity, which in turn led to root hypoxia and inhibition of root elongation. It may also be assumed that the use of mannitol or PEG in nutrient solutions have little relevance for natural drought conditions in the fields where drought conditions, and hence, plant perception appear more progressively allowing the plants to acclimate during several days. In order to discriminate between experiments that used moderate (coupled with maintained yield) or severe drought (associated with a yield reduction) and associated mechanisms (Figure 1, i.e., balance between C and mineral nutrients transported to the seeds), Table 2 presents the effect of drought (severe or moderate) on changes to seed nutrient contents in different species. In most species (except for soybean), a severe drought leads to an increased N concentration in grains (Table 2), which may be the result of a balance in favour of leaf N export relative to C, as opposed to a moderate drought that will less strongly affect photosynthesis.

The seed contents of other macronutrients like P, K, Mg, and sometimes Ca, are reduced most of the time (or remain unaffected) by severe drought (Table 2). The situation is more variable for Zn, Fe, Mn or Cu for which seed contents can be decreased or increased by severe or moderate drought, not only as a function of species but also for a given species subjected to different experiments (Table 2). In a hypothesis that parallels the one for N content, seed micronutrient contents are a result of a balance between C export from leaves and starch deposition in grains, and micronutrient leaf remobilization. For such micronutrients, the level of remobilization in leaves could be a function of only the vacuolar sequestration capacity and therefore dependent on root uptake (and storage) before anthesis. However, an extra supply of some micronutrients to the seeds could be provided by catabolism of polymers that may contain such micronutrients, and as a consequence of senescence induced by severe drought conditions. This could be the case for Zn, for example, which is associated with a large diversity of proteins (more than 1200 in *Arabidospis*) and for Fe, which is mostly stored in chloroplasts as ferritin. Mn and Ca are more difficult to understand because they are recognized as having little phloem mobility, and consequently a reduced leaf remobilization. However, it has been shown recently [32] that even though Mn and Ca can be efficiently mobilized from roots (probably through xylem vessels), they would require a significant mass flow, which is not compatible with the main effect of drought.

Other reasons may be invoked to explain changes in nutrient contents in seeds subjected to high or low drought severity, even though, to our knowledge, no literature is yet available. The ionomic content of vegetative tissue can be affected in a very unspecific way by mineral deficiencies or even by drought. The study by Maillard et al. [91] on the effect of individual nutrient deficiencies on the uptake of other nutrients in *B. napus*, found about 18 different situations where the uptake of other nutrients was increased. Such cross talk between nutrients was found, for example, during S and K deficiencies, which increased Mo and Na uptake, respectively. In both cases, this could be explained by the lack of specificity of some transporters that are up-regulated during deficiency. For example, sulfate transporters are mostly responsible for higher molybdate uptake under S deficiency, while K transporters, which are up-regulated by K deficiency, increased Na uptake. Similarly, Acosta-Gamboa et al. [92] showed that drought increases the leaf content of Mn and Cu, whereas it decreased Fe content and it was hypothesized that this was partly due to a higher requirement for Mn superoxide dismutase, which is involved in the ROS detoxification induced by drought stress. However, alternative explanations can be provided from [91] who showed that increases in Cu and Mn uptake in response to Fe deficiency can also be explained by the non-specific transport of these nutrients being shared by similar up-regulated transporters [93]. Ghandilyan et al. [67] highlighted that depending on the water availability, correlations between leaf nutrient contents were not necessarily maintained. In particular, correlations between K and Ca concentrations were positive under optimal water conditions, and negative under drought. Taken together, these results suggest that drought, while affecting root nutrient uptake may also modify cross talk between micronutrients (uptake and storage) and hence modify the composition of the leaf with potential consequences for seed composition.

A better knowledge of how ionomic composition of grain, and hence their quality, are modified by drought requires future experiments in which both drought conditions (repeated and moderated or severe) and mineral nutrient supplies are finely controlled under soil culture. A precise estimation of the modification of grain ionomic signature by drought should be done in relation to a kinetic analysis of leaf remobilization of each nutrient. Such experimental design was not previously used to our knowledge and should be considered. In such a way, it should be possible to distinguish (i) nutrients for which grain content is decreased as a result of limited leaf remobilization, from (ii) nutrient whose grain content is increased such as N (Table 2 and Figure 1), as a result of an efficient leaf remobilization coupled with a yield reduction and finally, (iii) nutrients that are indirectly increased in grain by drought as a result of nutrient crosstalk (see previous paragraph for examples). This would also require a better knowledge of all nutrient crosstalks induced by individual mineral deficiency as previously described [91] and for a larger range of plant species. This approach could also include the analysis of ionomic signatures combined with molecular analysis that would give access to metabolic pathways modulated by deficiencies and/or drought.

Moreover, the plant nutrient composition as well as the plant physiological response to drought are both genotype-dependent [67,83,92,94,95]. This emphasizes the need to take advantage of genetic variability, first to understand processes underlying micronutrient remobilization under drought and secondly to breed crops with higher seed micronutrient contents under water fluctuating environments. For the moment, strategies for enhancing drought stress tolerance by delaying senescence either via increasing chloroplast stability [96] or via induced production of CKs and the alteration in C and N homeostasis [97] have been tested and resulted in an increased grain yield, but could also lead to a limited N remobilization efficiency, implying a decreased protein content [54,98].

## 6. Conclusions and Perspectives

Under normal growth conditions, the storage and remobilization of macronutrients in particular have been extensively studied, but the handling of micronutrients remains less well known. Further, the mechanisms underlying nutrient remobilization to the seeds under drought conditions have been poorly addressed. In the context of human micronutrient malnutrition and climate change involving more frequent drought episodes, the need to breed for biofortified crops [99] adapted to water deprivation should become a major focus. Efforts are necessary concerning the understanding of micronutrient fluxes under limited mass flow due to decreased plant transpiration. These efforts may be coupled on the one hand with the screening of large collections of genotypes, and on the other hand with the exploitation of plant microbe interactions [100], which could enhance micronutrient availability, plant uptake, storage and remobilization to the seeds.

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