INTRODUCTION

Molybdenum is a trace element found in the soil and is required for growth of most biological organisms including plants and animals. Molybdenum is a transition element, which can exist in several oxidation states ranging from zero to VI, where VI is the most common form found in most agricultural soils. Similar to most metals required for plant growth, molybdenum has been utilized by specific plant enzymes to participate in reduction and oxidative reactions. Molybdenum itself is not biologically active but is rather predominantly found to be an integral part of an organic pterin complex called the molybdenum co-factor (Moco). Moco binds to molybdenum-requiring enzymes (molybdoenzymes) found in most biological systems including plants, animals and prokaryotes (Williams and Frausto da Silva, 2002). The availability of molybdenum for plant growth is strongly dependent on the soil pH, concentration of adsorbing oxides (e.g. Fe oxides), extent of water drainage, and organic compounds found in the soil colloids. In alkaline soils, molybdenum becomes more soluble and is accessible to plants mainly in its anion form as MoO$_4^{2-}$. In contrast, in acidic soils (pH <5.5) molybdenum availability decreases as anion adsorption to soil oxides increase (Reddy et al., 1997). When plants are grown under molybdenum deficiency, a number of varied phenotypes develop that hinder plant growth. Most of these phenotypes are associated with reduced activity of molybdoenzymes. These enzymes include the primary nitrogen assimilation enzymes such as nitrate reductase (NR), and the nitrogen-fixing enzyme nitrogenase found in bacteroids of legume nodules. Other molybdoenzymes have also been identified in plants including xanthine dehydrogenase/oxidase involved in purine catabolism and ureide biosynthesis in legumes, aldehyde oxidase (AO) that is involved in ABA biosynthesis, and sulfite oxidase that can convert sulfite to sulfate, an important step in the catabolism of sulfur-containing amino acids (Mendel and Haensch, 2002; Williams and Frausto da Silva, 2002). There are recent review articles on molybdoenzymes in plants, animals and prokaryotes (Mendel and Haensch, 2002; Williams and Frausto da Silva, 2002; Sauer and Frebort, 2003) that cover the extensive literature on the regulation and formation of Moco and the activity of Moco with molybdenum-dependent apoenzymes. Instead of re-examining this important component of molybdenum nutrition, this review will instead re-examine the effects of molybdenum nutrition in agricultural plants and explore the poorly understood aspect of molybdenum transport into and within the plant. In prokaryotes and lower-order eukaryotes, the molybdoenzymes are involved in nitrogen metabolism and the synthesis of the phytohormones abscisic acid and indole-3 butyric acid. Currently, there is little information on how plants access molybdate from the soil solution and redistribute it within the plant. In this review, the role of molybdenum in plants is discussed, focusing on its current constraints in some agricultural situations and where increased molybdenum nutrition may aid in agricultural plant development and yields.

Key words: Molybdenum, molybdate transport, nitrate reductase, Moco, *Vitis vinifera*, Merlot, Millerandage, sulfate transport, nitrogen fixation, nitrogen metabolism, plant nutrition.

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translated into an improved understanding of how eukaryotic systems transport molybdenum. This is not surprising as the primary molybdate transport systems present in prokaryotes are members of the ATP-binding cassette (ABC) protein superfamily. Members of this superfamily extend into plants; however, the numbers are large, where in arabidopsis alone there is predicted to be at least 129 putative proteins in the genome (Sanchez-Fernandez et al., 2001). Secondly a large number of other putative transport proteins that may encode molybdate transport systems still remain uncharacterized in sequenced plant genomes (Schwacke et al., 2003). Nevertheless, the prokaryotic systems are good starting points to discuss the types of eukaryotic systems that may exist and direct future research into specifically identifying plant molybdenum transport systems.

**AVAILABILITY OF MOLYBDENUM IN AGRICULTURAL SOILS**

Molybdenum is present in the lithosphere at average levels up to 2-3 mg kg⁻¹ but can increase in concentration (300 mg kg⁻¹) in shales that contain significant organic matter (Fortescue, 1992; Reddy et al., 1997). In agricultural soils, molybdenum is present as many different complexes depending on the chemical speciation of the soil zone. Mineral forms of molybdenum found in rocks include molybdenite (MoS₂), wulfenite (PbMoO₄) and ferrimolybdenite [Fe₂(MoO₄)] (Reddy et al., 1997). Release of molybdenum from solid mineral forms is through weathering, a process involving continual solution and oxidation reactions (Lindsay, 1979; Gupta, 1997). Dissolved molybdenum available to plants is commonly found in the soluble MoO₄⁻ anion form (Lindsay, 1979). Above pH 4-23, MoO₄⁻ is the common anion followed in decreasing order by H₂MoO₄⁻ > H₂MoO₄²⁻ > MoO₂(OH)²⁻ > MoO₂²⁻ (Lindsay, 1979). Once in solution, the MoO₄⁻ anion is subject to normal anion adsorption/desorption reactions, which are dependent on the specific chemistry of the soil solution. MoO₄⁻ can adsorb onto positively charged metal oxides (Fe, Al, Mn), clay minerals, dissolved organic compounds and carbonates. The adsorption of molybdenum onto positively charged metal oxides is strongly pH dependent with maximum adsorption occurring between pH 4 and 5 (K. S. Smith et al., 1997b). As the soil solution becomes more alkaline MoO₄⁻ availability increases. Every unit increase above pH 3, MoO₄⁻ solubility increases approx. 100-fold primarily through decreased adsorption of metal oxides (Lindsay, 1979). Consequently, the application of lime to agricultural soils has been an important tool to adjust soil pH and increase soluble molybdate.

Soluble MoO₄⁻ can also form ionic complexes with various ions in solution including Na, K, Ca and Mg, and can also be complexed with organic matter, particularly humic and fulvic acids (Jenne, 1977). The formation of these complexes can decrease the amount of MoO₄⁻ bound by metal oxides, increasing the amount of available MoO₄⁻ in solution (Reddy et al., 1997). Soil moisture also influences MoO₄⁻ availability where poorly drained wet soils (e.g. peat marshes, swampy organic rich soils) tend to accumulate MoO₄⁻ to high levels (Kubota et al., 1963). Many plants that grow under these soil conditions display high internal molybdenum levels, which can result in molybdenosis in ruminant animals if the material is used as animal feed (Scott, 1972; Gupta, 1997a). In contrast, well-drained sandy soils have been shown to leach significant amounts of applied molybdenum (Jones and Belling, 1967). The retention of molybdenum in sandy soils is very much pH dependent as acidic sands release negligible amounts of molybdenum in the leachate (Riley et al., 1987). Thus, soils rich in organic matter and with poor drainage traditionally accumulate soluble molybdate, while sandy soils are subject to molybdenum leaching but in a pH-dependent manner (Bloomfield and Kelso, 1973; Karmian and Cox, 1978; Riley et al., 1987).

**IDENTIFICATION OF MOLYBDENUM AS AN ESSENTIAL PLANT ELEMENT**

The requirement of molybdenum for plant growth was first demonstrated by Arnon and Stout (1939) using hydroponically grown tomato. Plants grown in nutrient solution without molybdenum developed characteristic phenotypes including mottling lesions on the leaves, and altered leaf morphology where the lamellae became involuted, a phenotype commonly referred to as ‘whiptail’ (Arnon and Stout, 1939). The only trace element that could eliminate these phenotypes was found to be molybdenum. The first reported case of molybdenum deficiency in an agricultural context occurred in mixed pasture grasses in the Lofty ranges of South Australia (Anderson, 1942). Local pastoralists reported significant failures of well-irrigated pastures containing subterranean clover (Trifolium subterraneum), perennial rye grass and Phalaris tuberosa. These pastures had been sown on sandy loam (ironstone) soils, which were low in nitrogen, slightly acidic (pH 5.5–6), rich in iron oxides and had received significant superphosphate treatments in previous years (Anderson, 1942, 1946). It was noted at the time that clover could grow in these soils after liming or when wood-ash was present (Anderson, 1942). It was later identified that molybdenum was the most abundant trace element present in the soluble and insoluble extractions of the wood-ash. Molybdate application at 2 lb per acre was capable of increasing lucerne yields approx. 3-fold over control plots (Anderson, 1942). Shortly thereafter, Davies (1945) and Mitchell (1945) demonstrated that the whiptail phenotype in cauliflower could be overcome with the addition of molybdenum to the soil. Walker (1948) observed that tomato grown in molybdenum-deficient serpentine soils could be rapidly rescued (return of green colour, loss of mottling) with application of sodium molybdate directly to the soil, or by leaf painting and leaf infiltration.

In contrast, molybdenum toxicity in plants under most agricultural conditions is rare. In tomato and cauliflower, plants grown on high concentrations of molybdenum will have leaves that accumulate anthocyanins and turn purple, whereas, in legumes, leaves have been shown to turn yellow (Bergmann, 1992; Gupta, 1997b). The greatest concern associated with high plant molybdenum levels is with crops used for grazing or silage production. Ruminant animals, which consume plant tissues high in molybdenum content,
can suffer from molybdenosis, a disorder that induces copper deficiencies (Scott, 1972). Fortunately this disorder can be controlled by directly maintaining adequate Mo/Cu ratios in the rumen diet or by altering the availability of molybdenum to plants by changes in soil availability (pH adjustment).

**VISUAL SYMPTOMS OF MOLYBDENUM DEFICIENCY IN PLANTS**

Molybdenum deficiencies have been documented in many plant species where phenotypes range in severity and appearance (Hewitt and Bolle-Jones, 1952a). In the Brassicaceae family, molybdenum deficiencies are strikingly pronounced and reproducible amongst many of its members. Visual effects in young plants include motting, leaf cupping, grey tinting, and flaccid leaves which are often found on seedlings that remain dwarfed until dying (Hewitt and Bolle-Jones, 1952a). In older plants, where deficiencies have been rescued or when deficiency levels are modest, the symptoms appear in younger leaf tissues with the characteristic loss of proper lamina development (whip-tail), leafy leaves and meristem necrosis (Hewitt and Bolle-Jones, 1952b). Investigation into the ultrastructure of leaves exhibiting whip-tail indicated that chloroplasts near the lesions became bulbous and enlarged with spherical protrusions bordered by chloroplast and tonoplast membranes (Fido et al., 1977).

Deficiency symptoms can also be masked by the indirect effect of molybdenum on nitrogen assimilatory enzymes (i.e. NR). Many horticultural, cereal and legume crops growing at deficient molybdenum levels in the presence of nitrate fertilizers will develop pale green leaves and, at times, necrotic regions at leaf margins with accompanied decreases in overall plant growth (Hewitt and Bolle-Jones, 1952a; Agarwala et al., 1978; Chatterjee et al., 1985; Chatterjee and Nautiyal, 2001). Molybdenum-deficient oat and wheat develop necrotic regions on leaf blades, and seeds are poorly developed and shrivelled (Anderson, 1956; Chatterjee and Nautiyal, 2001). In maize, molybdenum deficiency shortens internodes, decreases leaf areas and causes the development of chlorotic leaves (Agarwala et al., 1978). In reproductive tissues in maize, molybdenum deficiency can alter the phenotypes in developing flowers, including delayed emergence of tassels, small anthers, poorly developed stamens, and reduced pollen grain development (Agarwala et al., 1979). Pollen that is released from the anthers has been shown to be shrivelled and have poor germination rates (Agarwala et al., 1978, 1979). In grapevines, molybdenum deficiency has recently been suggested as the primary cause of a bunch development disorder called Millerandage or ‘hen and chicken’ (Williams et al., 2004). Millerandage (Fig. 1) is characterized by grapevine bunches that develop unevenly, where fully matured berries are present in a bunch alongside a large number of fertilized underdeveloped berries as well as unfertilized swollen green ovaries (Mullins et al., 2000). Millerandage has been reported primarily in *Vitis vinifera* ‘Merlot’ but unpublished anecdotal reports have suggested the problem also occurs in Cabernet Sauvignon and Chardonnay cultivars (P. Dry, The University of Adelaide, Adelaide Australia, pers. comm.). In Merlot vines displaying Millerandage, other characteristic molybdenum-deficiency responses also appear including shortened zigzag-shaped internodes, pale-green leaves, increased cupped and flaccid leaves, and marginal leaf necrosis (K. Gridley, University of Adelaide, unpubl. res.).

**BIOCHEMICAL RESPONSE IN PLANTS TO MOLYBDENUM DEFICIENCIES**

Molybdenum deficiency affects plant metabolism at many different levels. The responses are strongly linked to the requirement of molybdenum for the various types of molybdenum enzymes present in plants. Plant molybdenum enzymes can be

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**Fig. 1.** Incidence of Millerandage in *V. vinifera* ‘Merlot’ and recovery from after foliar molybdate treatment and/or grafting onto rootstocks. Millerandage is identified by altered bunch development where berries within bunches at final harvest are at different developmental stages including fertilized matured ripened berries, fertilized but poorly developed berries and unfertilized enlarged green ovaries. (A) Merlot bunches at harvest displaying Millerandage in the (–Mo) treatment versus normal bunches in the (+Mo) treatment. (B) Final berry yields in response to foliar molybdenum treatments pre-flowering. Merlot vines were grown on own roots or grafted onto the rootstocks Schwarzmann and 140 Ruggeri.
Molybdenum deficiencies are primarily associated with poor nitrogen health particularly when nitrate is the predominant nitrogen form available for plant growth. Inability to synthesize Moco will reduce the activity of the critical nitrogen-reducing and assimilatory enzymes including NR and XDH (Agarwala and Hewitt, 1954; Spencer and Wood, 1954; Afridi and Hewitt, 1964, 1965; Randall, 1969; Jones et al., 1976; Agarwala et al., 1978). In most plant species, the loss of NR activity is associated with increased tissue nitrate concentrations and a decrease in plant growth and yields (Spencer and Wood, 1954; Agarwala et al., 1978; Chatterjee et al., 1985; Unkles et al., 2004). Accordingly, in spinach plants grown under molybdenum-deficiency conditions, leaf NR activity was found to be reduced and overall final plant yields lower than control plants grown on adequate levels of molybdenum (Witt and Jungk, 1977). In wheat, molybdenum starvation was also shown to reduce maximum NR activities (lower potential VMAX) irrespective of the regulatory control of NR by light and dark periods (Yaneva et al., 2000). Re-supplying molybdenum as a foliar spray or in supplemented nutrient solution in most instances will readily recover NR activity (Spencer and Wood, 1954; Afridi and Hewitt, 1964; Jones et al., 1976; Witt and Jungk, 1977). In the wine grapevine Vitis vinifera ‘Merlot’, poor growth during establishment and variable yields in mature plants grown in many South Australian vineyards is positively correlated with reduced petiolar molybdenum levels (Williams et al., 2004). Preliminary experiments by Ngaire Brady and colleagues (unpubl. res.) have demonstrated NR activity is significantly depressed in both Merlot shoots and roots even when grown with nutrient solution containing nitrate-N and adequate amounts of sodium molybdate (Fig. 2). It is believed that this is not the result of a mutation in the NR apoenzyme or in Moco biosynthesis as Merlot is capable of nitrate reduction when molybdenum is applied as a foliar treatment. Painting molybdate directly onto a leaf will induce NR activity in the treated leaf and in untreated leaves elsewhere in the canopy (Fig. 3). From this preliminary study, it would indicate the phenotype present in Merlot is not related to the synthesis and activity of Moco (Mendel and Haensch, 2002) or the NR apoenzyme but most likely associated with a disruption in the mechanism controlling molybdenum uptake and or internal redistribution in the xylem and or phloem. Interestingly, NR activity can also be rescued and plant growth returned to a ‘normal’ state by grafting Merlot onto hybrid North American rootstocks (Fig. 1). From this phenotype it would suggest the mutation in Merlot rests with its inability to readily accumulate molybdate from the soil solution.

Molybdenum and its regulation of symbiotic nitrogen fixation

The other notable influence of molybdenum on plant nitrogen metabolism is in nitrogen-fixing legumes. The symbiotic bacterial enzyme nitrogenase is comprised of two subunits one of which is the MoFe protein directly involved in the reduction of N2 to NH3. Supply of molybdenum and
Fe to bacteroids is therefore an important process and most likely a key regulatory component in the maintenance of nitrogen fixation in legumes. Molybdate supplied by the plant must traverse nodule cellular membranes (plasma membrane and the peribacteroid membrane) as well as the bacteroid outer and inner membranes to reach the bacterial nitrogenase complex. A modABC transport system is most likely involved in bacteroid molybdate uptake; however, currently there is no information on the mechanism controlling molybdate transport into nodules and across the peribacteroid membrane. What is known, with respect to molybdenum and legume nitrogen fixation, is that molybdenum supply increased (Brodrick and Giller, 1991). XDH activity has been shown to increase when phytopathogenic fungi infect both cereals and legumes. Whether this response is aimed at oxidative defence mechanisms it still unknown; however, in pea, XDH activity is strongly correlated with the activity of superoxide dismutase (Pastori and Rio, 1997). How this and other plant defence-related responses are linked to plant molybdenum nutrition is poorly understood. There is little direct evidence to conclude that improvements in plant molybdenum levels results in a decrease of disease, with the exception of small number of studies which indicate molybdenum fertilization can improve resistance to verticillium wilt in tomato (for a review, see Graham and Stangoulis; 2005). However, as discussed by Graham and Stangoulis (2005), this response may just be through improved plant health and not a direct effect on molybdenum in the defence response.

Molybdoenzymes not associated with nitrogen metabolism

Molybdoenzymes are also involved in the synthesis of the phytohormones ABA and indole-3-acetic acid (IAA). The Moco-dependent AO, catalyses the final steps in the conversion of indole-3-acetaldehyde to IAA, and the oxidation of abscisic aldehyde to ABA. Mutations in either the AO apoprotein or enzymes involved in Moco biosynthesis and Moco activation (sulfuration) will disrupt ABA synthesis (Marin and Marion-Poll, 1997; Schwartz et al., 1997; Sagi et al., 2002; Hesberg et al., 2004). Low ABA levels result in plants with a wilt appearance through excessive transpiration and loss of stomatal control, altered seed dormancy, and impaired defence responses (Mendel and Haensch, 2002). It has been shown recently the ABA-deficient mutants flacca and aba3, which both show wilt phenotypes, are disrupted in the Moco sulfuration step, which is required to activate the inserted Moco in AO (Bittner et al., 2001; Sagi et al., 2002). One of the distinct phenotypes in molybdenum-deficient Merlot is flaccid and cupped leaves similar to that observed in flacca and aba3 (Robinson and Burne, 2002).
2000). More research is required to ascertain whether AO activity in Merlot is affected by molybdenum deficiencies and the wilty phenotype associated with AO activity and sufficient ABA production.

MOLYBDENUM TRANSPORT

The mechanism(s) controlling molybdenum transport in plants and all higher-order organisms are still unknown. To date, molybdenum transport systems have only been identified and characterized in prokaryotes (bacteria) and some lower order eukaryotes (Self et al., 2001; Mendel and Haensch, 2002). In bacteria, the molybdenum transport system consists of multiple transport systems that ensure effective transfer of molybdenum into the cell. From studies in *Escherichia coli*, three systems are known to exist (Fig. 4), a primary high-affinity ABC-type transport system (ModABC) (Maupin-Furlow et al., 1995) and two secondary systems including an ABC-type sulfite transporter and a non-specific anion transporter (Maupin-Furlow et al., 1995; Self et al., 2001). Each of these proteins is encoded from genes found on a single operon (Maupin-Furlow et al., 1995; Walkenhorst et al., 1995). Downstream of the ModABC operon are two individual operons containing the regulatory genes ModE and ModF (Grunden et al., 1996). In many other bacteria and Archaea, Mod operons with similar or altered composition to that of *E. coli* have been identified through genome sequence homology (Grunden and Shamugam, 1997; Self et al., 2001). However, only a few have been genetically and functionally characterized including Mod genes present in *Azotobacter vinelandii*, *Staphylococcus carnosus* and *Rodobacter capsulatus* (Luque et al., 1993; Wang et al., 1993; Neubauer et al., 1999).

ModABC consists of three proteins including a periplasmic molybdate-binding protein (ModA), an integral membrane channel protein (ModB) and an energizing protein (ModC). Molybdate binds to ModA (Grunden and Shamugam, 1997; Self et al., 2001). However, only a few have been genetically and functionally characterized including Mod genes present in *Azotobacter vinelandii*, *Staphylococcus carnosus* and *Rodobacter capsulatus* (Luque et al., 1993; Wang et al., 1993; Neubauer et al., 1999).

Fig. 4. Molybdate transport systems in *E. coli*. (A) Molybdate transport in *E. coli* is considered to involve three systems. The modABC protein complex, a sulfate transport complex similar to CYS UWA and an unidentified nonspecific anion channel. (B) Diagram of the mod operon present in *E. coli*. 

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Molybdate transport into plants

Since there is no known molecular mechanism controlling molybdate transport in plants, and higher organisms for that matter, we are left to speculate on the types of systems based on the information we have from prokaryote and whole-plant molybdenum nutrition studies. Unfortunately, linking prokaryotic molybdate transport systems to the processes, which occur in eukaryotes, is not direct as there is limited sequence homology to modABC, modE and ModF in either arabidopsis or rice genomes or any other large plant expressed sequence tagged collections or partially sequenced genomes. However, there are similarities in physiological responses to molybdenum between prokaryotic and eukaryotic systems, namely the close interaction with sulfate transport. Sulfate is a similar-sized anion to molybdate, and evidence from prokaryotic studies suggests that sulfate transport systems and selenate-sensitive anion channels are capable of molybdate transport (Self et al., 2001). Stout and Meagher (1948) first demonstrated that, in tomato, molybdate (99Mo) uptake in simple single salt buffer was significantly enhanced in the presence of phosphate and inhibited with sulfate. In a more representative nutrient solution where both phosphate and sulfate were present, sulfate was still found to be an effective competitor to molybdate uptake (Stout et al., 1951). In contrast, 99Mo uptake into tomato increased when phosphorus was withheld from the nutrient solution which could be quickly reversed with phosphorus re-supply (Heuwinkel et al., 1992). From this study, it would appear molybdate is bound and transported across the plasma membrane using a phosphorus transport system. However, firstly, the competition studies demonstrated that when phosphorus levels were adequate, low concentrations of molybdate failed to effectively compete with phosphorus and, secondly, accumulated molybdate did not quickly move from roots to shoots and was instead readily available for exchange with non-labelled molybdate (Heuwinkel et al., 1992). These data suggest the phosphorus transport system may effectively bind and accumulate molybdate but would appear to have limited impact on molybdate transport under good growing conditions where the soil has adequate amounts of available phosphorus. It is also interesting to note that sulfate accumulation was significantly repressed during the phosphorus starvation period (Heuwinkel et al., 1992), a result which strengthens the case for the involvement of sulfate transport systems in molybdate transport. Since the initial observation by Stout and Meagher (1948), sulfate has since been shown to be an effective regulator of molybdenum uptake in many plants under a wide range of growing conditions (see review by Macleod et al., 1997). The similar size of the two anions and the relative concentrations in the soil solution most likely contribute to the competition observed with sulfate. However, the effect of sulfate on molybdate uptake is not solely at the root/soil interface. Soybean plants showed decreased molybdenum levels in aerial parts of the plant as the sulfate supply increased (Sing and Kumar, 1979) even if molybdenum was applied as a foliar spray (Kannan and Ramani, 1978).

The influence of other ions on molybdate uptake is poorly understood. In excised rice roots, the uptake of molybdate (0.01 mM) was significantly enhanced in the presence of 0.1 mM FeSO4 but not in FeEDDHA (Patel et al., 1988). Interestingly, in free-living cowpea Rhizobium grown in iron-deplete conditions, the addition of high concentrations of molybdenum (1 mM) results in a release of a siderophore which appears to bind molybdenum and influences its uptake into the cell (Kannan and Ramani, 1978). Molybdate is highly mobile once in the plant where foliar absorption and translocation occur quickly. Williams (2004) showed that foliar-applied molybdate was rapidly distributed throughout the plant, including translocation towards the stem and roots within 24 h. Work completed by Ngaire Brady and colleagues (unpubl. res.) showed that foliar application of molybdate onto V. vinifera ‘Merlot’ restored NR activity in non-treated leaves elsewhere in the plant canopy (Fig. 3). Indeed, Brodrick and Giller (1991a), have shown good plant growth responses from foliar molybdenum application in the field. The mobility of molybdenum in plant tissues does appear to be genetically
controlled. Brodrick and Giller (1991) observed different molybdate partitioning patterns between two Phaseolus vulgaris cultivars. One variety had a distinct advantage in distributing molybdate to developing seeds, nodules, roots and pod walls (Smith et al., 1995).

**PUTATIVE PLANT MOYLBDATE TRANSPORTERS**

The close interaction between molybdate and sulfate transport in many biological systems suggests a similar transport system is likely involved in the movement of molybdenum into and within plants. The first plant sulfate transporters (SHST1, SHST2, SHST3) were identified from sulfur-starved roots of the tropical forage legume Stylosanthes hamata (Smith et al., 1995). The SHST(1–3) clones were identified by their ability to functionally complement a yeast sulfate transport mutant YSD1 (Takahashi et al., 1996, 1999, 2000; F. W. Smith et al., 1997; Bolchi et al., 1999; Vidmar et al., 1999; Hawkesford, 2003). Since then a number of sulfate transport systems has been genetically identified and characterized in plants including genes from arabidopsis, barley, maize, potato, soybean and wheat (Hawkesford, 2003). In arabidopsis, there are 12 identified sulfate transporters with significant sequence homology and two more which are more distantly related (Hawkesford, 2003). This rich gene collection in many plant species has enabled distinct groups to be identified based on their sequences, cellular localization and response to sulfate (Takahashi et al., 1999). Group I sulfate transporters are high-affinity systems (K_M 1-5–10 μM) primarily expressed in roots, and increase or decrease in expression in response to sulfur starvation or supply, respectively. Group II sulfate transporters are considered low affinity systems (0-1–1-2 μM) based on their functional properties when expressed in yeast cells. Group II transporters also respond to sulfur starvation through increased expression levels. Group III transporters are mainly expressed in leaf tissues and account for five of the 14 sulfate-like transporters identified in arabidopsis. For the remaining two groups there is less information on their functionality in plants. Initial reports indicated a member of group IV (AtSultr4;1) may be targeted to chloroplasts (Shibagaki et al., 2002), while group V members are distantly related to members of group I–IV and no functional experimentation has been completed on them. The role of the sulfate transporter family in plants is slowly becoming clearer. Recently, the arabidopsis AtSultr1;2, which is a member of the group I sulfate transporters, was shown to be involved in sulfate uptake in planta where a T-DNA lesion in the AtSultr1;2 locus allowed plants to grow on toxic concentrations of selenate and reduced its ability to accumulate sulphate into root tissues. There is an obvious requirement for more research into identifying the in planta function of the remaining sulfate transporters in plants before any of them can be nominated as putative molybdate permeases. However, one avenue of research that could be explored further is the role of these transport proteins when expressed in heterologous expression systems such as yeast cells. Although significant headway has been made in identifying genes encoding sulfate transport proteins very little information exists on the functional properties of most of these transporters in relation to anion selectivity, pH regulation and kinetic activities. Early studies in yeast demonstrated selenate and chromate as effective inhibitors of sulfate uptake (Breton and Surdin-Kerjan, 1977). Thus, selenate has been an effective screening tool to identify mutants that have disruptions in sulfate transport (Smith et al., 1995; Cherest et al., 1997).

Using a selenate-resistant mutant YSD1, the selectivity of this mutant for sulfate transport and other anions such as molybdate is being explored. By removing molybdate from the media by activated charcoal scrubbing it has been possible to demonstrate that molybdate uptake at low external concentrations is also impaired in the yeast mutant (K. Gridley, unpubl. res.). This low molybdate media screen has been incorporated into ongoing experiments where selected plant sulfate transporters are being expressed in yeast and ranked on their ability to rescue growth on reduced molybdenum concentrations.

**CONCLUDING REMARKS**

Molybdenum nutrition is an essential component to healthy plant growth. Molybdate which is the predominant form available to plants is required at very low levels where it is known to participate in various redox reactions in plants as part of the pterin complex Moco. Moco is particularly involved in enzymes, which participate directly or indirectly with nitrogen metabolism. However, Moco is also uniquely involved in ABA synthesis where it has a significant effect on ABA levels in plant cells and consequently a role in water relations and transpiration rates through stomatal control and in stress related responses. There is significant scope in exploring practices, which optimize molybdenum fertilization in crops where nitrate is the predominant available N source or in nitrogen fixing legumes. There is also a large gap in the understanding of how molybdate enters plant cells and is redistributed between tissues of the plant. For instance the mechanism controlling molybdenum transport to nitrogen fixing bacteroids may be a unique control mechanism by which the plant can regulate the symbiosis indirectly through molybdenum availability to support nitrogenase activity. From our recent work with the grape-vine cv. Merlot, we are starting to appreciate the influence of molybdenum on plant development and better understand mechanisms, which may be responsible for molybdenum uptake from the soil. It is ironic that it took a new industry to be expanded in South Australia where molybdenum first made its mark as an essential plant element to again reinforce the importance of molybdenum in plant development. Much more research is required to ascertain the simple processes involved in how plants gain access to molybdenum and how the element may be used in the future to expand growing areas where soil molybdate profiles limit plant growth.

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