







#### **Review Article**

# Molybdenum potential vital role in plants metabolism for optimizing the growth and development

Muhammad Shoaib Rana<sup>1,2</sup>, Parashuram Bhantana<sup>1-3</sup>, Muhammad Imran<sup>4</sup>, Muhammad Hamzah Saleem<sup>5</sup>, Mohamed G Moussa<sup>1,2</sup>, Zaid Khan<sup>5</sup>, Imran Khan<sup>1</sup>, Mufid Alam<sup>6</sup>, Muhammad Abbas<sup>7,8</sup>, Rana Binyamin<sup>9</sup>, Javaria Afzal<sup>1,2</sup>, Muhamad Syaifudin<sup>1,2</sup>, Intisar Ud Din<sup>1,2</sup>, Muhammad Younas<sup>1,2</sup>, Ilyas Ahmad<sup>10</sup>, Md Ashrafuzzaman Shah<sup>1,2</sup> and Chengxiao Hu<sup>1,2\*</sup>

<sup>1</sup>Key Laboratory of Arable Land Conservation (Middle and Lower Reaches of Yangtze River), Ministry of Agriculture, Micro-elements Research Center, College of Resource and Environment, Huazhong Agricultural University, Wuhan 430070, China

<sup>2</sup>Hubei Provincial Engineering Laboratory for New Fertilizers, Huazhong Agricultural University, Wuhan

<sup>3</sup>Agricultural Research Station, Pakhribas, Dhankuta, Nepal, Agricultural Research Council, Nepal

<sup>4</sup>Department of Crop Science and Technology, College of Agriculture, South China Agricultural University, Guangzhou 510642, PR China

<sup>5</sup>MOA Key Laboratory of Crop Ecophysiology and Farming in the Middle Reaches of Yangtze River, College of Plant Science and Technology

 $^6$ National Key Laboratory of Crop Genetic Improvement, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

<sup>7</sup>Nuclear Institue of Agriculture, Tandojam, Pakistan

<sup>8</sup>Institute of Agriculture Resources and Regional Planning (IARRP), Chinese Academy of Agricultural Sciences (CASS), Beijng, China

<sup>9</sup>University of Agriculture Faisalabad Sub Campus Burewala Vehari, Pakistan

<sup>10</sup>Soil and Water Testing Laboratory for Research, Multan, Pakistan

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\*Corresponding author: Chengxiao Hu, Professor, Key Laboratory of Arable Land Conservation (Middle and Lower Reaches of Yangtze River). Ministry of Agriculture, Micro-elements Research Center, College of Resource and Environment, Huazhong Agricultural University, Wuhan 430070, China, Tel: (+977) 9849991998; +86 27 87288730; E-mail: hucx@mail.hzau.edu.cn; hailstormsea@gmail.com

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#### Abstract

Molybdenum importance for appropriate plant functioning and growth is inconsistent by the most of the plants in respect to the total quantity that is obligatory for them. Molybdenum is a micronutrient that is directly involved in the metabolic functions of nitrogen in the plant. The transition metal molybdenum, in molybdate form, is essential for plants as a number of enzymes use it to catalyze most important reactions in the nitrogen acclimatization, the synthesis of the phytohormone, degradation of the purine and the detoxification of the sulfite. There are more than known 50 different enzymes that need Mo, whether direct or indirect impacts on plant growth and development, primarily phytohormones and the N-metabolism involving processes. On the other hand, in the synthesis of ABA uniquely Moco is involved, there on the level of ABA Moco effect is highly vital and ultimately by the response in the stress and the stomatal control, it has a very important role in the rate of transpiration and water relations. The practices that are involved in the fertilization of Mo optimization in crops, has a very important scope in discovering and improving these practices where the legumes are fixing the N or NO<sub>3</sub> is primarily source of available N. The deficiency of Mo and to enhance the molybdoenzymes activity, it may be very effective and vital important to use the spray of Mo as foliar application through the soil. The most recent understanding that from the soil how the plant gets access Mo or how they redistribute it is not still clear. However in the system f prokaryotes, it has been found that in plants it has likewise physiological Mo transport phenotypes. So, the mechanism of transport of Mo in the prokaryotes is needed as well as the reconsideration of anion transport mechanism that is in plants, will provide a help to solve that how this is accumulated. In this review, the discussion covers about the vital importance of Mo to enhance the productivity for optimizing the yield concentrating on metabolism, uptake

#### Introduction

Molybdenum (Mo) is very important and an essential micronutrient for plants, animals and bacteria [1–3]. A lot of soils in the world suffer due to the deficiency of microelements such as Mo [4,5]. Mo is deficient in more than 44.7 million hectares of land and B is deficient in 33.3 million hectares in China [6]. Consequently, the deficiency of Mo and B of soil is an extensive agricultural problem that induces quality and yield losses in various crop species worldwide [6,7]. Mo-deficient plants show poor growth [8] and less contents of chlorophyll and ascorbic acid [9].

It is seen that itself Mo not so much biologically active but mostly occurs as a vital part of a complex organic pterin that is also being called molybdenum co-factor (Moco). Including plants, prokaryotes and animals, Moco being found to binds with molybdoenzyme in the most of the biological systems [10]. A lot of different phenotypes starts to develop when under the insufficiency of molybdenum plant are being grown. Due to the reduction of molybdoenzyme activity maximum of these phenotypes are associated. Enzymes most of them include that are involving in prime N-assimilation like the nitrogenase (nitrogen-fixing enzyme), Nitrate Reductase (NR) and those that are present in legumes nodules bacteroids. In plants together with xanthine dehydrogenase/oxidase some other additional molybdoenzyme also has been recognized that have very important role in the ureide biosynthesis and purine catabolism. It can be seen that in the legumes, during the biosynthesis of ABA the conversion of sulfite to sulfate may be carried by the sulfite oxidase and Aldehyde Oxidase (AO), that is very significant in amino acid metabolism that contains sulfur [10]. Current review articles about the molybdenum in plants, prokaryotes and animals have shown the wide-ranging literature on formation and regulation of Moco and its activity with molybdenum-dependent apoenzymes [10-12]. In the lower order eukaryotes and prokaryotes, the system about the molybdate transport is characterized very well and designed at biochemical, genetics and physiological levels [13]. In the plant development aldehyde oxidase (AO; EC1.2.3.1) that is plant molybdoenzyme, has a very important role in relation to the development of plant and stresses in the environment [14-16]. Xanthine dehydrogenase and molybdenum-hydroxylases

aldehyde oxidase in plants have distinctive reactive oxygen species signatures that are tempted by the abscisic acid and drought. Biosynthesis of phytohormones is catalyzed by the AO multigene family members in the preceding step, such as ABA and IAA by converting abscisic aldehyde and indoleacetoaldehyde to their respective phytohormones [12]. IAA being the vital member of auxins that are plant hormones has a major crucial role in a lot of activities of plants comprising abscission, root initiation, phototropism, fruit development, gravitropism and apical dominance [12]. The involvement of IAA to stress as in salinity and deficiency of water in plants has also been suggested. In response to environmental stress ABA also plays a very crucial and important role in plants [17].

As molybdenum is vital constitute in the nutrition of plants, this review will inspect the transport of Mo within and into the plants and will also explore the crucial and comprehensive nutrition of Mo in plant growth and development.

#### Impact of Mo insufficiency on Growth

Molybdenum insufficiency resembles nitrogen deficiency, as in the plant metabolism the most significant role of Mo is the reduction of nitrate. Plants facing the Mo insufficiency, the leaves start to become pale, restricted in growth, flower development and formation may also be affected and eventually wither. The most typical visual deficiency impact in dicotyledons is the severe abnormality in size. These are caused by the inadequate discrepancy of the vascular bundles at initial development stages of leaf and the necrosis in tissue [18].

There is a direct relationship between the molybdenum bioavailability in the soil and the molybdenum contents in soil. The molybdenum availability will be low if a soil having the lower soil pH [18] and the plant that is facing the Mo less availability shows the lesions and leaves different morphology was 1st time explained by Arnon and Stout, [19] and then in a comprehensive way by Hewitt's group [20].

Mo insufficiency could also be due to a mutation in the Mo-specific uptake system [21,22]. In the MOT1, the knockout mutants in mitochondrial transport revealed that there is very minute change in the growth pattern [23]. As is being seen that though, in plant cells there are numerous Mo transporters,

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but a severe phenotype has not been described yet. Finally, when there will be any kind of imperfection of biosynthesis of Moco that can cause molybdenum insufficiency and by this all the activity of Mo-enzyme is highly decreased or can be lost pleiotropically that has severe consequences for the cell. The loss of Mo-enzyme is most drastic for the plant?

- 1) It has been known that the NR-mutants can destruct the complete NR-activity
- 2) If as a solitary N source, with No<sub>3</sub>- these mutants cultured then loss of NR-activity is lethal [24].
- 3) There is no observable phenotype if a smash in one of the two mARC proteins occurs.
- 4) Although somewhat, but it is not intensely altered plant phenotype as the XDH1 loss has no such as lethal consequence.
- 5) For AO, in AAO3 only communicated a mutation, so any disorder or loss of AAO3 will ultimately cause a wilty phenotype and it will cause a severe effect on plant growth and development as when abscisic aldehyde converted into ABA, in this AAO3 has a very crucial and important role [25,26]
- 6) High sulfur dioxide to an atmosphere has not challenged as no phenotype seen provided if there is loss of SO [27].
- A wilty phenotype can be observed if the absence of AO activities occurs because basically ABA reduction was observed due to the insufficiency of Moco-sulfarase (ABA3) [28].

So, Moco has very important role in plant growth and development and plant will come toward the death situation if Moco is lost.

## Molybdenum in soils

Mo generally exists in highly soluble form, and is rare in the soils building it liable to leaching. Though, usually consider that in the acidic soils the molybdenum is attached to mineral surface, it may avoid the leaching, but it may also obstruct uptake by microbes. Usually contents of Mo between 0.6 and 3.5 ppm exist in the most of the agricultural soils with about 2.0 ppm an average Mo and 0.2 ppm, average available molybdenum. As an oxycomplex (MoO<sub>L</sub><sup>2-</sup>), Mo largely occurs in the soil. Because of this, in its behavior in the soil, Mo more resembles to the phosphate or sulfate. By soil minerals and colloids molybdate is adsorbed in related way to these two anions. This adsorption is very closely dependent on the soil pH [29]. It increases as the pH falls but at the neutrality it is very low. On acid soil availability of Mo for the plants is the poorest and can be upgraded by foliar application, seed priming, seed coating pelleting and with liming, in case soil is not characteristically lacking in molybdenum [18].

## Molybdenum adsorption in soils

The pH dependency of Mo adsorption on the soil resembled

the one found for clay and oxides minerals. pH is a very dominant factor in the availability of nutrients [30-32]. It controls many processes occurring in the soil system [33-35]. Absorption demonstrated a peak in the range of pH 3-4, and then declined with the increasing pH above 4. With temperature, there is an little increase in the adsorption of Mo on surface of soil [36]. Carroll, et al. [37], to examine the transport of Mo in soil with and without biosolid amendment employed the miscibledisplacement and batch-equilibrium experiments. Sorption of the Mo was revealed to be greater for the biosolid-amended soil. Though, Mo related with biosolid-amended soils is comparatively mobile and bioavailable. Adsorption of Mo was probed in California on 5 arid zone and the results showed that adsorption of molybdenum from pH 4-8 salinity solution was independent as a function of pH of solution [37]. By using soil chemical properties Mo adsorption was predicted: iron oxide content, organic carbon content, cation exchange capacity and inorganic carbon content. Generally, in the acidic oxidizing soils, trace metals are readily available and are less available in neutral reducing or alkaline soil )[38,39]. Moreover, not like the other trace metals, with increasing soil pH, there is always increase in availability of Mo in higher plants [40]. In generally, in its anionic form (MoO42-) mainly, Mo becomes accessible to plants and soluble in soils having alkaline properties. By contrast, Molybdenum obtainability in acidic soils declines as the adsorption of anion increases to soil oxides (pH<5.5) [41] Figure 1.

Montmorillonite < nontronite < metahalloysite and illite < kaolinite < kaolinite, the relative adsorption on the clay minerals increased in this order. Goldberg, et al. [43], determined the magnitude of adsorption of Mo following as increasing order: kaolinite < illite < montmorillonite. Though, comparability is difficult about the adsorption affinity per unit surface area or per unit mass, since between adsorbents in different experiments the suspension density varies. They reported that on clay mineral Mo adsorption near the pH 3 was more and then rapidly declined by the increase in pH up to the adsorption become almost zero near pH of 7. The kaolinite point of zero charges of was moved to a high acid pH value for Mo adsorption on kaolinite, demonstrating mechanism an inner-sphere adsorption for the Mo on such surfaces. Including adsorption at inner or outer-sphere complexes and precipitation, adsorption can occur through a variety of mechanisms.

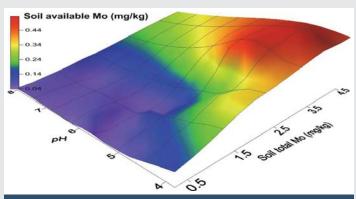


Figure 1: Rational Graphs; soil Mo, pH and available Mo [42].

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To study the mechanism of Mo adsorption with iron oxides, Lang, et al. [44] used pressure-jump relaxation in order to evaluate the special effects of aging on complex Mo/goethite formed during Mo chemisorptions and to explain Mo/goethite interaction. They concluded that the within suspension Mo transport resulted the slow relaxation, which does not depend on temperature and the fast relaxation represents Mo chemisorptions to the goethite, which decreases with increasing temperature. Understanding the surface speciation in subsurface and surface environment bioavailability is critical to calculating on mineral surfaces. Arai [45] to investigate in-situ Mo surface speciation on goethite applied near edge structure analysis the X-ray absorption. Experimental results showed that with a decline in pH Mo (VI) coordination environment changes from the tetrahedral to octahedral. The inner-sphere surface species formed by the predominant tetrahedral molecules of Mo (VI), near-neutral pH however at pH 3-4, comprising octahedrally and tetrahedrally a surface specie mixture are present. In the soil-water environment, Prediction of processes of Mo transport, the surface speciation of Mo (VI) the Ph dependent multinuclear may be important.

In sulfidic environments, thiomolybdate species,  $MoO_xS_{4-x}$  are formed in conditions, anoxic (i.e.,  $DO < 1 \text{ mg l}^{-1}$ ) [46]. This conversion of goethite could be due to the reduction facilitated by the activities of microbial [47]. Impact in the acidic soil is due to the strong adsorption in relation between the pH and molybdenum that can not be ignored. Some other soil properties may also effect on the availability of Mo. A detail of factors that influence the availability, leaching and adsorption process are shown in Figure 2.

A role of tetrathiomolybdate (MoS<sub>1</sub><sup>2-</sup>) in the chemical pathway may be in anoxic sediments, fixation of Mo. Its incorporation was adsorption on or co-precipitation with iron sulfides [48-50]. It forms relatively stable complexes when once MoS,2- reacts with pyrite that are engaged as Mo-Fe-S "cubane" structure irreversibly [29,51,52]. Cuboidal Mo-Fe-S clusters are formed by Mo on pyrite that has been shown by X-ray spectroscopy [51]. The adsorption of the MoO, 2- and MoS<sub>1</sub><sup>2</sup>, goethite and pyrite onto these two main iron minerals, has been talked to explain the probable mechanisms of the immobilization of Mo in the anoxic conditions [29], and have concluded that even in the existence of, sulfate, silicates and phosphate, the MoS<sub>1</sub><sup>2-</sup> may be an eventual pool and may also regulator Mo enhancement in sediments,. The results of the experiment presented that adsorption of MoO<sub>2</sub> and MoS<sub>2</sub>. is a sturdy function of surface loading and pH. The retention of molybdate, in µmol/g, with the iron minerals followed the increasing order of the  $MoO_{\lambda}^{2-}$  – pyrite <  $MoS_{\lambda}^{2-}$  – pyrite <  $MoO_{\lambda}^{2-}$ -goethite < MoS<sub> $\lambda$ </sub><sup>2-</sup> -goethite. Helz, et al. [50], recommended that FeS ion activity products may control the molybdenum concentrations, as well as the pH in euxinic basins. On the Mo adsorption, some of other anions showed little effect; i.e., selenite adsorption onto γ -Al2O3 [53,54], arsenate adsorption onto clay minerals [43] and silicate onto goethite and pyrite Figure 3 [29].

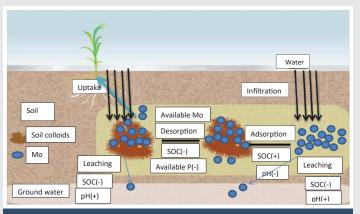
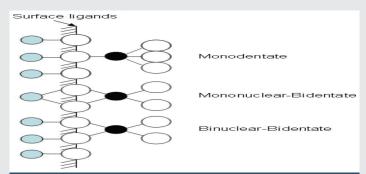


Figure 2: Mo leaching, adsorption and influencing factors.



**Figure 3:** Simplified schematic diagrams of possible molybdate adsorption complexation modes on metal oxides. Legend: () Oxygen, () Molybdenum, () Metal atom (i.e., Al or Fe)[126].

# Oxidation and redox behavior of Mo species

Molybdenum resembles tungsten and vanadium, in its chemical properties, the Group 5th first number, rather than Cr. Molybdenum, chemically is tremendously versatile, forming compounds in array of the freely interconvertible states of oxidation. Molybdenum shows all the oxidation states from 2- to 6+ in its compounds, among which the lowest oxidation states, ranges from 2--1+, found in the complexes with the ligands acceptor, primarily cyclopentadiene, C-monoxide and related compounds, P, nitric oxide and Ar-donor ligands. Molybdenum the short oxidation states (2- to 2+) are unlikely be to arise in enzymatic processes or encountered in biological systems. In its oxidation states the Mo in its oxidation 3+ to 6+ forms the majority of complexes with S-, O-, halogens and N-donor ligands. Mo (IV) can also be stabilized by S in which the most stable sulfide is disulfide and in complex ion [Mo(CN) 8]4-, Mo (IV) is powerfully stabilized by cyanide. By the oxo molybdenum species, Mo(V) and Mo(VI) in oxidation states are dominated. Bard, et al. [55], have reported that molybdates are less powerful oxidants in acidic solutions than chromates and vanadates where the oxidation potential decreases with the increasing pH. These species of molybdenum are coordinated with hydroxide, oxygen, sulfate, water and chloride, with respect to their oxidation and the reduction [56]. However, lower oxidation states of Mo than 3+ and 4+ are not obtained by reduction, while in alkaline and neutral solutions by dithionite molybdates (Mo VI) may be reduced to molybdenum(V) and oxo molybdenum(IV) complexes, except that weak reducing agents, including, sulfide and hydrogen make Mo blue.

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Mo chemistry in the soils is very complex because the Mo oxidation state differs from II to VI. Molybdenum concentrations within the soil profile and species vary depending on the nature of the soil adsorptive complexation and with the chemical characteristics of soil solution [57]. Under anoxic conditions Mo is predicted to be in the IV oxidation state [58]. Predominantly Mo is found as Mo (VI) oxyanions ( $MoO_4^{2^-}$ ) with a pH of 4–8 in oxic soils. The chemistry of Mo in soils is further complicated by the complexation of Mo with metals (e.g.,  $CaMoO_4^0$ ,  $MgMoO_4^0$ ) [59]. In natural water Molybdenum forms molybdate Mo(VI) oxyanions. Mo(VI) anions' adsorption in acidic soils and sediment typically control the fate and transport of Mo(VI) anions in the natural environment of water-soils-sediments.

## Plants, enzymes need Mo and Mo cofactor

In order to have biological activity, Mo is complexed with pterin compound thus making the prosthetic group named Moco, [15,60,61]. As anion MoO<sub>4</sub><sup>2-</sup>, Mo is abundant in oceans. Moreover, molybdate ion is the form that is only available for bacteria and plants in soil. If the plant takes too much high Mo, it may have toxic effects on it [62]. On the opposite side its insufficiency is lethal for the plant. Only a handful of Molybdenum-enzymes were found among eukaryotes while, most they are of bacterial origin.

To scavenge Mo in the existence of competing anions molybdenum needs specific systems oof uptake. Mainly, transport follows from up to down to the stem and roots signifying when molybdate applied solely to leaves, that the molybdate is very mobile translocated among numerous tissues of the plant. Moreover, the result displays that an effective inhibitor of molybdate uptake is sulfate pioneered speculations on the nature of molybdate transporters [63].

Table 1. In higher plants components of molybdenum metabolism (Arabidopsis thaliana). In fact, molybdate and sulfate as they are alike in size, have a high grade of resemblance, both possess the structures tetrahedral and the double negative charge (MoO, 2-, SO, 2-,). Biosynthesis of Moco is the only process known to need molybdenum straight by plants that is finalized in the cytosol and initiated in the mitochondria [16,64]. Only little knowledge is present about the enzymatic regulation and genetics of Moco biosynthesis in the plants, even though the biosynthesis of Moco is well understood. The information that is existing only in this reverence concerns in the Arabidopsis MOT1 mutant, molybdenum metabolism-related genes expression that was developed to induce the deficiency of molybdate endogenous by the supply additional molybdate was grown without it. [65]. In fact, under molybdate deficiency, the genes CNX2 and CNX6 Moco biosynthesis were revealed to be the up-regulated, which was similarly true for the Moco sulfurase ABA3, the molybdo enzymes NR2 and NR1, and transporter MOT2. Molybdenum cofactor (Moco). In plants Mo-enzymes to this end known are five in number.: mitochondrial amidoxime reductase (mARC), xanthine dehydrogenase (XDH), N-reductase (NR), S-oxidase (SO) and aldehyde oxidase (AO). There is another type of Mo-containing cofactor, in addition to the pterin type of the cofactor, another type of cofactor having Mo, calling in bacterial nitrogenase which is found only once in nature, developing the cofactor iron-molybdenum, FeMoco. Biological nitrogen fixation in the biosphere is a vital step in the cycle of N, nitrogenase is required for Biological nitrogen fixation. All other containing Mo, considered to this end have the pterintype cofactor Table 2 [66].

Generally, the transmission of an oxygen atom to is catalyzed by all the Mo-enzyme or to from a substrate (Hille, 2002). Each reaction, by transfer of two electrons either oxidation or reduction, is also characterized by this, which

Table 1: In higher plants components of molybdenum metabolism (Arabidopsis thaliana).

Protein names	Agi code	Known / proposed function		
MOT1/SULTR 5;2	AT2G25680	Molybdate transport		
MOT2/ SULTR5;1	AT1G80310	Molybdate transport/export from the vacuole		
CNX1	AT5G20990	Moco biosynthesis step 3		
CNX2	AT2G31955	Moco biosynthesis step 1		
CNX3	AT1G01290	Moco biosynthesis step 1		
CNX5	AT5G55130	Moco biosynthesis step 2		
CNX6	AT2G43760	Moco biosynthesis step 2		
CNX7	AT4G10100	Moco biosynthesis step 2		
Nia1/NR1	AT1G77760	Nitrate reductase (Minor form)		
Nia2/NR2	AT1G37130	Nitrate reductase (Main form)		
SO	AT3G01910	Oxidation/elimination of cytotoxic sulfite		
mARC1/ MOSC1	AT4G44720	Unknown		
mARC2/ MOSC2	AT1G30910	Unknown		
AAO1	AT5G20960	Unknown		
AAO2	AT3G43600	Unknown		
AAO3	AT2G27150	ABA biosynthesis		
AAO4	AT1G04580	Synthesis of benzoic acid		
AtXDH1	AT4G34890	Purine degradation		
AtXDH2	AT4G34900	Unknown		
ABA3/LOS5	AT1GI6540	Mocosulfuration and activation of AO and XDH proteins		
ATM3/ABCB25	AT5G58270	Transporter involved in cytosolic Fe-S assembly and Moco synthesis		

Table 2: Mo enzyme in the plant (Arabidopsis thaliana), subcellular location and their function.

Mo enzyme	Number of genes	Subcellular location	Function	ROS/RNS side product
Nitrate reductase	2	Cytosol	Nitrate assimilation	NO
Sulfite oxidase	1	Peroxisome	Sulfite detoxification	$H_2O_2$
Xanthine dehydrogenase	2	Cytosol	Purine degradation, NADH oxidase	Superoxide anions
Aldehyde oxidase	4	Cytosol	Synthesis of ABA (Auxins)	H <sub>2</sub> O <sub>2</sub> , Superoxide anions
Mitochondrial amidoxime reductase	2	Mitochondria	Detoxification (?)	n.d
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imposes to fluctuate between IV and VI, its oxidation state of Mo atom. Mo-enzymes are the homodimeric proteins that are present in eukaryotes which are functioning solitary as a dimer, but not as monomer. On the monomer of enzyme isolated domain are identified that are bound to it, different prosthetic groups are involved by some harbor an electron transport chain. Figure 4, point out the five Mo-enzymes of plants domain structure where by the regions that are very much capricious in the order, the domains are linked as a result clearly serving as inter-domain joint regions. It is evident that Mo-enzymes into two classes can be subdivided: XO oxidase family is symbolized by AO, and XDH while Mo-enzyme's SO-class is formed by the NR and SO both classes sharing the domains. From GTP to cPMP the conversion starting, in mitochondria basic steps of Moco biosynthesis are shown, entirely in cytosol succeeding steps continue. Moco-binding proteins (MoBP) and Moco biosynthesis enzymes (named Cnx) have been represented in blue. By Cnx5, consisting of Cnx6 and Cnx7 MPT-synthase is sulfurated, with sulfur donor that is primary (X-S) militarized by Cnx5 (RLD) rhodanese-like domain being unidentified. For adenylation and activation of the small MPT synthase subunit Cnx7, the adenylation domain of the Cnx5 (AD) is required. It is supposed that after dithiolene formation, copper (Cu) is inserted directly. Moco can be either bound to these Mo-enzymes NR, SO, XDH, mARC and AO, to a MoBP (Moco-binding protein), or to the MBD (Mocobinding C-terminal domain) of Mocosulfurase ABA3. ABA3 C-terminus is sulfuration platform for Moco. ABA3 domain like NifS makes the persulfide protein-bound, which bound to its C-terminus is transferred to the Moco that for the sulfurated, Moco exchanges the non-sulfurated. In the figure, Mo-enzyme monomers domain structure is given. It is obvious that Moenzymes of eukaryotes are interrelated to each other: A pair is formed by the AO and XDH, from ABA3-MBD, mARC arose and forms NR a pair with SO Figure 5 [16].

## Mo crucial role in biological nitrogen fixation

Specifically for plants, Mo is a micronutrient that with nitrogen-fixing bacteria form root nodules, however trace amounts of Mo are also used in a protein involved with

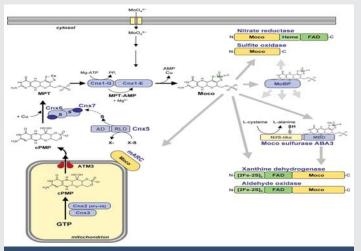
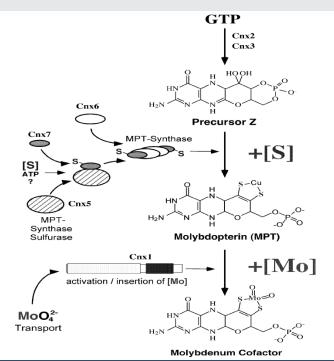


Figure 4: In plants biosynthesis, maturation and distribution of Moco [16].



**Figure 5:** Formation of molybdoprotein and insertion of sulfur, conversion of GTP to precursor Z, the formation of Moco and insertion of molybdenum. According to the Cnx nomenclature the protein involved is named.

nitrogen metabolism and uptake plants that do not form nodules [67]. Its significance to the N2 fixation is vibrant, given that Mo in 'FeMoCo' cofactor is at heart of the nitrogen reduction process - at least for the most of the nitrogenases. Two atoms of molybdenum contained by the Mo-Fe protein and has two distinct types of oxidation-reduction centers: four Fe-S centers and two iron-molybdenum cofactors called FeMoco. The active site of Mo-containing nitrogenase protein constitutes by the FeMoco of the nitrogenase in the N<sub>2</sub>-fixing organisms [68]. Although at low supply, into the nodules molybdenum is preferentially transported. [69], molybdenum deficiency-induced nitrogen deficiency is widespread in legumes depend on N<sub>2</sub> fixation, predominantly in acid mineral soils of the subhumid and humid tropics. It is also described that an impaired nitrogen fixation activity showed by B. japonicum strain deficient in Mo transport when inoculated to the soybean roots. More dramatic signs of deficiency in laboratory conditions showed by the several different legumes that were sternly starved of Mo [70,71]. There are reports that in the field conditions foliar applications of the molybdenum to grain legumes increase the levels of nodule mass and N<sub>2</sub> fixation, consequentially in higher seed yield and N Figure 6 [72,73].

Soybean growing without Mo fertilizer on a Mo-deficient soil in the upper left. Soybeans were a normal green color, indicating adequate N. Soybean without Mo but with N fertilizer as shown in the upper right. Soybean growing without N in the lower-left fig. and with Mo caused in superior growth and effectively nodulated soybean plants while nodulated soybean roots from plants growing on Mofertilized soil in the lower right [74].











Figure 6: Note the apparent N-deficiency symptoms exhibited by the leaves. Soybean growing without Mo fertilizer on a Mo-deficient soil in the upper left. Soybeans were a normal green color, indicating adequate N. Soybean without Mo but with N fertilizer as shown in the upper right. Soybean growing without N in the lower-left fig. and with Mo caused in superior growth and effectively nodulated soybean plants while nodulated soybean roots from plants growing on Mofertilized soil in the lower right [74].

## Molybdenum uptake, storage and transport into the cells

By the sulfate transporters or related systems, it is being proposed that molybdate distribution and import are facilitated [75]. In contrast to the homeostasis of bacterial molybdate, the transport of Mo in eukaryotes is less understood. Protein MOT1that belongs to the family that is large sulfate carrier and that was revealed to transport molybdate by the ultrahigh affinity across the cellular membranes [23,76,77]. In the plasma membrane surprisingly, it was not found to reside. Conflicting reports localized it to the mitochondrial envelope or to endomembrane system [23,77]. As in the cytosol, into the Moco-backbone the insertion of Mo occurs both suggested the subcellular locations are questionable. Another molybdate transporter of sulfate transporter family, in addition to MOT1, it is being described the vacuole provide functional evidence for MOT2 that is confined to the tonoplast and as an important molybdate store [21,22], and low content of Mo in the leaves while in seed by the accumulation of Mo contents, the plants that are deficient in MOT2 are characterized. Still for Mo cellular importer is missing, but it is being expected that this task is carried not only in animals but also in autotrophs by the additional transporters. It can be assumed, in addition to a high-affinity system, non-specifically molybdate can also move in the cell by sulfate uptake system that has been revealed for a sulfate transporter [78-80].

In the wildtype and MOT2 mutant leaves, however, it is found total molybdenum contents with the levels of Moco were to correlate, which indicates molybdate at cellular levels, Moco synthesis is adjusted by the plant.

## Molybdenum efficacy enhancement techniques

Seed coating, treatment and pelleting with Molybdenum: Numerous studies have shown the efficacy of the Mo seed coating [81,82]. Seeds were treated by the Mo (80 g/ha) causing in improved comparative grain yield, chlorophyll index, seed weight and pod number. It shows the better results when soybean seeds with ferrous sulfate 500 mg/kg and ammonium molybdate 250 mg/kg were pelleted were highly effective for the improvement of dry matter production, yield, plant height, growth rate and area index of leaves [81,82]. Similarly in field experiments, to increase yield of soybean and cowpea on acidic soils, benefits from the Mo applied together with the rock phosphate or alone, were greater or comparable than liming evaluating the effectiveness of a number of pelleting materials [83]. Though, from Mo seed-coating, several reports indicating no toxicity or improvement. Burton and Curley [84] reported that bacterial survival, N fixation and nodulation were strappingly suppressed when seed with sodium molybdate was pelleted. By inoculant and Mo nearly inoculated bacteria 99% died just after seed treatment in four days. As groundnuts kernel yield is increased due to seed dressing nearly more than 300 kg ha-1 in Senegal by applying 28 g ammonium molybdate/ ha ([85]. In Sierra Leone on groundnuts grown on an upland soil seed pelleting with 0.2, 0.4 and 0.8 g inoculation with rhizobia plus sodium molybdate/100 g seed was tested. Where sodium molybdate was applied at 0.2 g/100 g was applied to seeds, by this it is seen an increased protein content by 4.21%, DM yield by 17.2%, 14.0% grain yield and N uptake 38.5% [86]. Rhodes and Nangju [83] led field trials in increasing yields of cowpea and soya bean to estimate the efficiency of the numerous pelleting materials on an acid soil in the Sierra Leone. Mo increased yield and the growth of the cowpeas when it is applied combined with the rock phosphate or either alone, but on soya bean it had no significant effect on the yield and growth, even though per plant number of nodules increased significantly. It is reported by the Rhodes and Kpaka [87], by application of Mo, it boosted dry matter, seed yield and pod weight of cowpeas. At rate of 0.4 g/100 g seed treatment, yield increased by pelleting seed with nitro-molybdenum by 21%, over control or 1.39 t ha-1 (Table 3). Applying Mo by this method may show attraction to the small landholding farmers as it is cheap, simple, is less subject to the vagaries of wind and rain and does not require any spraying equipment.

Some other researchers also observed on bradyrhizobium, salts suppressive effects used as molybdenum sources [88,89]. But there is also reports present that indicates in improving crop performance Mo seed coating is effective, from the bacterial strains used for inoculation, it may have toxic effects. So, before using Mo seed coating, it must be evaluated the seed coating efficacy of Mo with bacterial strains.

Table 3: Effects of Mo on cowpea performance [86].

Teatment	Dry matter (g/ plant)	Nodule weight (mg/plant)	Pod weight weight (t/ha)	Seed yield (t/ha)		
No Mo	10.47a	144.0a	1.445a	1.15a		
Mo sprayed	13.09b	146.3b	1.519a	1.15a		
Mo pelleted	13.56b	179.0a	1.892b	1.39b		
LSD (0.05)	2.08	49.3	0.22	0.18		

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## Seed priming with molybdate

Numerous studies pointed out that for Mo application, seed treatment is a more effective method than soil application. For instance, in eastern India 48 trials conducted, mean yield was increased by 17%-22% by application of Mo as compared to the control where Mo was not applied and when it is applied to soil the increase was 20%-25% ([90,91]. Similarly, over the no application control, Mo through the seed treatment (4 g kg-1 seed) application was more economical and effective for increasing the yield by 15.79%; 10.53% with 1.5 kg/ha the soil application [92]. 0.1 or 1% sodium molybdate solution, priming of Trifolium subterraneum L. (Subterranean clover) seeds in improving yield and growth was as alike effective as through the soil application, in soil that is deficient in Mo. Donald and Spencer [90] reported that in grains high N and Mo content resulted in the seed priming than soil application as the application levels increases for the maximum yield. Mohandas [93] reported that improved nitrogen fixation, dry matter accumulation, yield and nodulation was seen when common bean seeds primed in sodium molybdate. It has been reported that a yield increase of compared with Mo soil application 27% by seed priming in a pot study on chickpea, with Mo for 8 h at 0.5 g/L solution of sodium molybdite, while in the field experiment, increase in the yield of chickpea from the same treatment was 20% [94,95]. In Bangladesh, over untreated control yield an increase was 37%-90% and was up to 50% more than the water-soaked control in trials at farmers' fields at different locations [96]. By adding rhizobium in the priming solution, the efficacy with Mo seed priming could be enhanced. Similarly, in Vigna radiata L. (Green gram), priming with the rhizobia and sodium molybdate ominously enhance the nutrient uptake, nodulation, crop yield, nitrogen fixation and growth of the plant. In fulfilling the Mo requirements of various crops, the Mo application by the priming of seed is highly economical and more effective as compared to the soil application, however with rhizobia its amalgamation needs to be the more investigated and modified. Pattanayak, et al. [97], indicated that fertilizer application efficiency is also increased by combining the seed treatments that indicate the better use of the resources. Even though in the priming media incorporation of rhizobial strains was synergistic, the protocol should be modified for micronutrient requirements and individual crops Table 4.

## **Foliar application**

Katyal and Randhawa [99] indicated that a spray solution may be comprised of 0.1%-0.3% of soluble Mo. With the increasing soil pH, the Mo availability increases, and acid soils that have a pH <5.2, Mo available amount to plants is very low, i.e., 0.10 mg/kg-0.25 mg/kg [18]. Since in the phloem and xylem Mo is highly mobile, and this Mo by seed treatment can be provided or may be by the foliar application as the crops require it in low amounts. Foliar sprays of Mo are more effective often than the applications to soils, principally for the acidic soils, and if it is applied at early stages of plant development it may be of supreme effectiveness [100]. Foliar spray effectiveness depends on nutrient its translocation into the plant and uptake rate by the leaves [101]. Mo applied by leaf spray, the leaves rapidly absorb it. Campo and Hungria [102] found that to the soybean nodules translocation of the Mo was very rapid, and after application of five days reported the highest concentration of molybdenum in nodules.

On enhancing plant yield the effect of Mo fertilization is often related to an improved capability of the plant to consume nitrogen. Biscaro, et al. [103] confirmed that grain yield of common bean enhances when there is the Mo and N combined leaf supply. By plants Mo status the activities of nitrate reductase and nitrogenase are affected, and in the plants that are suffering from the deficiency of Mo their activities are often suppressed [104]. After 25 days plant emergence Mo application through the foliar application (40 g/ha<sup>-1</sup>) greatly improved the NR and activities of nitrogenase that results in increase in total nitrogen accumulated in the shoots of common bean [73]. calengo, et al. [105] reported that in common bean deficiency of Mo results in low efficiency of N assimilation of plants.

Lombin, et al. (1985) [106] considered the effect of molybdenum i.e,30 g ammonium molybdate/ha, in 1971, 1972 wet and dry year respectively, sprayed on groundnut three weeks after sowing at the Samaru, northern Nigeria Table 5.

It has been found that Mo in the dry year is more likely to increase kernel yields of the groundnut short-season varieties. The groundnuts kernel yield by 200 kg ha<sup>-1</sup> increased by the foliar application of Mo [107]. After 10 days emergence100

Table 4: Effect of molybdenum seed treatment in different crops on yield of grain and grain enrichment.

Source	App. mode	App. rate	Crop	Increase in grain yield over untreated control (%)	Increase in nodule number	content over untreated	Reference
Sodium molybdate	Seed treatment	0.2g/100g seed	Groundnut	14			Haque and Amara [86]
Sodium molybdate	Seed treatment	0.4g/100g seed	Soyan bean	154			Haque and Bundu [98]
Nitro-molybdenum	Seed treatment	0.4g/100g seed	Cowpea	21			Rhodes and Kpaka [87]
Sodium molybdate	Seed soaking	1 mg L <sup>-1</sup> , 1h	Common bean	12.66	122.73	-	Mohandas [93]
Sodium molybdate	Seed soaking	2 mg L <sup>-1</sup> , 1h	Common bean	53.68	272.73	-	Mohandas [93]
Sodium molybdate	Seed soaking	5 mg L <sup>-1</sup> , 1h	Common bean	11.61	90.91	-	Mohandas [93]
Sodium molybdate	Seed priming	0.0026 M, 8h	Chickpea		-	7400	Johnson, et al. [122]
Sodium molybdate	Seed priming	0.0026 M, 12h	Lentil		-	0	Johnson, et al. [122]

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Table 5: Effect of Mo application on groundnut yield.

Year	Variety	Plant part	Pod yield(Kg/ha			
	Vallety		No Mo	With Mo	Difference	
1971	0.61	Kernel	1718	1894	+176	
	S.61	Haulm	2078	2892	+814	
1972	S.61	Kernel	1416	1293	-123	
	5.01	Haulm	2992	3633	+614	
1972	Spanish 205	Kernel	1893	2298	+405	
1972	MK 374	Kernel	1922	2095	+173	

g sodium molybdate/ha foliar sprays applied to maize that eliminated the Mo-deficiency symptoms in Zimbabwe. Though, when the spray was applied at the same rate after 4 weeks of emergence, that proved ineffective. While it has been seen that on maize crop when we use the foliar application at 100 g / ha sodium molybdate after 10 days of emergence, it was seen that was adequate to raise Mo content in seed above (0.08 ppm Mo) critical concentration [108]. In leaf tissue of sunflower, the increase of N and NH,+ and concentration with Mo rates shows that N assimilation improved by this Mo that is, reduction of NO, to NH, and consequently transformed in proteins, other compounds that are organic and amino acids in plants. Firstly all the nitrogen should be reduced to NH, when it is taken up by plant, because for assimilation into the N carrying amino acids, the only reduced nitrogen form that is available to plants [109,110].

# Soil application

There are many crops, that need Mo fertilizers [111]. High nitrogen application and under the low pH conditions with the Mo, oilseed rape should be fertilized. The Mo uptake by oilseed rape can also be decreased by the sulfur application [112]. Usually, 70–200 g Mo ha<sup>-1</sup> for the forage legumes is adequate and for the other field crops. In soil in absence of molybdenum, the plant molybdoenzymes can be broken down and brutally obstruct the nitrogen fixation by the soil bacteria [22].

Up to 400 g/ha cauliflower may need molybdenum but among the crops and soils, optimum dose for Mo varies. Katyal and Randhawa,1983 reported that cauliflower may need Mo application every year but one application on fodder and pasture plants may create for several years, residual effects. For about 1 to 15 years residual effects may last in Australia and New Zealand, where arable crops and pasture are receiving with super-phosphate molybdenum-enriched giving Mo 20-60 g/ ha, [113]. Parish, et al. [114] indicated the significantly improved sugar-cane growth were to a low humic Latosol Mo applied in Mauritius. In the West African semi-arid zone Molybdenum applications on sandy aeolian soils had positive effects on N fixation, growth, seed yield, nodulation of groundnuts [85]. A positive response toward the growth of cowpea with Mo in an experiment was seen on acidic Shante soil at Ogbomosho, which was typical of soils that are sandy in nature in the transition zone of the Nigerian forest/ savanna and at Ibadan on Adio soil. Mo application at the rate of 0.05 ppm shows the more efficient response, with up to 4ppm Mo smaller responses occurring at this application rates. To the Mo deficiency in acid soils maize appears to be relatively susceptible.

On very acidic soils in Australia, some research conceded out that indicated that when fertilization of Mo applied it was possible to attain higher yields of wheat. In the seed material the actual Mo concentration shows the increment in yield. Better the yield; lower the Mo content in seeds, stimulating Mo fertilization effects [111]. Mo rates between 35 and 140 g/ha had a noteworthy effect. Towards seed molybdenum increasing concentration in common bean has been examined by applying the Mo rates at high level, between 90 and 720 g/ha, applied to leaves [115].

In Zimbabwe greenhouse experiments indicated on a clay-loam red soil Mo deficiency as a major growth-limiting factor. Field experiments show that when we apply Mo in the maize, then early growth of maize increased on the soils having a pH between 4.4 and 4.8 [116–127].

#### **Conclusion**

Our considerate of the function and the biological role of the molybdenum is progressing rapidly. Moco as it occurs in CNX mutants the complete loss of it is lethal and when they are grown in the soil leads to the death of plants. However in the cell culture, when grown on the media these mutants could be retained alive with the reduced N as N-source. As well as Moco biosynthesis proteins the crystallization of further molybdenum enzymes is under way. Though Moco on ABA levels have an important effect in the plant cells, Moco is also uniquely takes part in ABA synthesis and as a result a role in transpiration rates and water relations through in the stressrelated responses and stomatal control. In crops the practices which optimize the fertilization of Mo have significant scope in exploring where the predominant available N source is No.- or in N fixing legumes. To predict the Mo geochemical transport in subsurface and surface environments, the distribution and sources of the Mo species of MoS,2-and MoO,2- in an aqueous solution are critical information. The significant components, comprising clay, oxides, Fe, iron sulfide, Al, , organic matter and manganese oxides in soils and bed sediments, show different adsorption capacity to Mo.

In our understanding, in plants functions and role of Mo, recent years have conveyed rapid improvement. Obviously, the research concentrates both on the studying relationships of structure-function of the Mo-enzymes and on detailed enzymology of the Moco biosynthesis.

#### **Future prospects**

For NR there is a lot of physiological data, but not for SO, AO and XDH and there are, yet, a number of open questions.

- I. By member of the AO family what the additional metabolic reactions are catalyzed?
- II. Why SO localized in the peroxisomes?
- III. What is the exact role of SO?
- IV. For the MPT synthesis what protein donates the copper?
- V. In the plant defense and reactive oxygen metabolism, what is the role of XDH?

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- VI. Will by the molybdopterin ligand metals other than tungsten or molybdenum be found to be coordinated?
- VII. After its release during the Moco formation what protein accepts copper?
- VIII. The CnX4 role is not so much clear, what key roles it has? The molybdate transporter how it is organized? what gene code for molybdate transporter?
- IX. For the Moco biosynthesis organized how is the multienzyme complex?
- X. For the Moco to meet with regards to changing demands of the cell, how Moco biosynthesis is being regulated?
- XI. For the Moco insertion, what is the mechanism behind this that is involved in apo-enzymes?
- XII. In some enzymes, what factors influence the incorporation of the tungsten instead of the molybdenum?
- XIII. What role is being played by the Cu in the synthesis of Moco?
- XIV. Furthermore, about factors very diminutive is known that are regulatory the interaction of proteins and expression of genes involved in the homeostasis of molybdenum, thus needing advanced comprehensive analysis on metabolome, transcriptome, ionome level, proteome and interactome.

Requires further investigation, the involvement of each cofactor scaffold to the complete chemistry of given enzyme, that the numerous scaffolds represent corridors generating sulfur-containing chelators that can control, trap and activate the transition elements as catalysts. With specialized functions in the eukaryotes some of proteins are the precursors of these, such as ubiquitin-like protein conjugation 64 and the G-protein-based signaling; others, such as the gephyrin, that is crucial for synaptogenesis, have gathered the additional functions.

The coming years perhaps into these will bring deep insight into innovative molybdenum aspects within the physiological and metabolic network of cells. In the field of molybdenum enzymes future research is likely to focus on detailed mechanistic of the functions of cofactors, the cofactor biosynthesis and cofactor allocation in specific enzymes.

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