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Research Article

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Effects of Sodium Chromate Metal Stress on Carbohydrate Content and Enzymatic Inhibition in Horse gram (*Dolichos biflorus* L).

Dr. Kota Jagpal
Head & Asst. Prof of Botany
Department of Botany, Govt Degree College Chennoor-504202

Abstract

This study investigates the impact of sodium chromate stress on carbohydrate metabolism and enzymatic activity in *Dolichos biflorus* L., an important leguminous crop. Under increasing concentrations of sodium chromate, a reduction in total sugars, starch, and non-reducing sugars was observed, while reducing sugars increased in treated plants. Specifically, total sugars decreased by 16.04% and starch content by 18% at the highest concentration of 3.0 mg/ml sodium chromate, compared to control plants. Non-reducing sugars were significantly affected, showing a 66.1% reduction, whereas reducing sugars increased by 36%. Additionally, the NRS/RS ratio showed a marked decrease of 66.6%, indicating a shift in carbohydrate balance under metal stress.

The observed changes are likely linked to the inhibition of phosphoglucomutase, an enzyme crucial for sucrose synthesis, as well as other enzymes involved in carbohydrate metabolism. Previous studies support that fluoride, and heavy metals can inhibit enzyme function, disrupting glucose-6-phosphate conversion and thus lowering sucrose production. Our findings align with similar responses in other plant species exposed to metal stress, demonstrating that sodium chromate significantly alters carbohydrate fractions and interferes with enzyme activities vital for metabolic processes in *D. biflorus*. This study highlights the need for understanding metabolic adaptations in crops under heavy metal stress, with implications for crop resilience and environmental stress management.

Keywords: Heavy metal stress, Sodium chromate, Carbohydrate metabolism, Enzymatic inhibition, *Dolichos biflorus* L., Phosphoglucomutase

1. Introduction

Heavy metal accumulation in plants is a complex process influenced by both the beneficial and toxic properties of various metals. Plants require metals like copper (Cu), cobalt (Co), iron (Fe), molybdenum (Mo), manganese (Mn), nickel (Ni), and zinc (Zn) for their growth and metabolic functions (Hänsch & Mendel 2009). Trace amounts of other metals, including arsenic (As), cadmium (Cd), mercury (Hg), and selenium (Se), are also beneficial but only in very low concentrations (Rascio & Navari-Izzo 2011). For example, Cu supports photosynthesis and electron transport, but excessive Cu and Cd in the soil can disrupt processes like germination and root development in certain plants such as Solanum melongena (Neelima & Reddy 2004). High levels of these metals lead to oxidative stress, reducing plant growth and health (Levitt 1980; Stadtman & Oliver 1991).

Plants employ different mechanisms to tolerate, accumulate, or exclude metals in polluted environments. Some plants act as indicators, showing metal accumulation levels; others are accumulators, storing metals in tissues, while excluders limit metal uptake to protect themselves (Knight et al. 1997). For example, *Thlaspi caerulescens* (Brassicaceae) and *Viola calaminaria* (Violaceae) can detoxify accumulated metals within cells (Baker 1981). In contrast, species like Festuca rubra stabilize contaminated soil by preventing erosion without excessive metal uptake (Wong et al. 1994).

Hyperaccumulators actively transport metals to shoots and leaves, achieving concentrations significantly higher than non-accumulators (Baker & Brooks 1989). These plants offer a potential tool for phytoremediation, an eco-friendly approach to soil decontamination that retains soil structure and allows recovery of pollutants (Cunningham et al. 1995; Salt et al. 1994). Phytoremediation can mitigate chromium (Cr) pollution, a serious issue globally due to industrial waste. High Cr levels, particularly hexavalent chromium (Cr (VI)), are toxic and cause various health problems, notably in industrial workers (Sujana & Rao 1997). In soils, Cr exists in multiple oxidation states, with Cr (VI) posing the greatest threat due to its solubility and mobility in water (Nriagu & Nieboer 1988).

The ongoing increase in chromium usage is concerned due to its environmental impacts, including forest cover reduction and pollution in ground and surface waters. In India, chromium contamination from industries has caused groundwater levels of Cr to exceed safe limits by a significant margin, affecting human health and ecosystems (Sujana & Rao 1997; Tirazon 1992). Phytoremediation and metal-tolerant plant species hold promises for addressing such contamination, offering a sustainable alternative for soil and water recovery.

1.1 Plant description:

Dolichos biflorus, commonly known as horse gram, is an annual herb growing to a height of 1 to 1.5 feet. It has a sub-erect, twining habit, and is covered with villous hairs. The stem is slightly angular, hairy, and typically pigmented purple, as are the petioles. The leaves are alternate, stipulate, and pinnately trifoliate, with each leaf having three ovate, entire leaflets with an acute apex. The leaflets are herbaceous, villous, and slightly oblique laterally.

The inflorescence consists of one to three flowers in the leaf axils, exhibiting a purple hue. The flowers are papilionaceous, with five polypetalous petals in a descending imbricate arrangement, and a characteristic purple eye on the standard. The flowers are zygomorphic, hypogynous, and pentamerous, with a campanulate calyx and five gamosepalous sepals. There are ten stamens arranged in a diadelphous (9+1) pattern, and the ovary is superior, monocarpellary, and unilocular, with marginal placentation. The style is terminal, curved, with a capitate, hairy stigma.

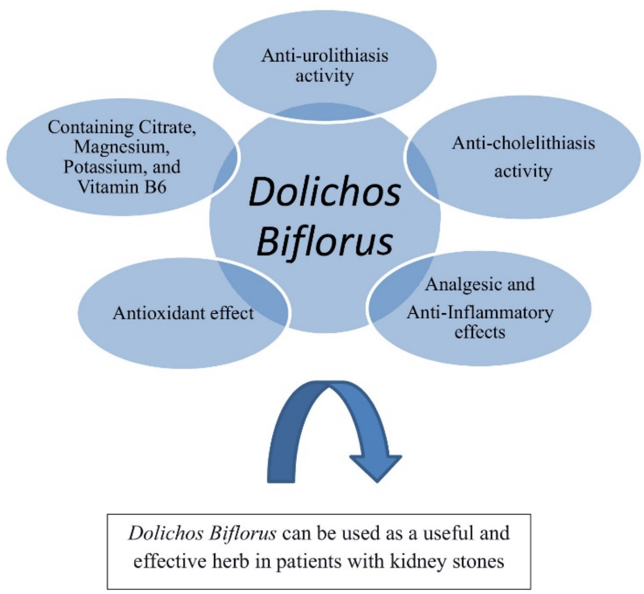
The plant typically self-pollinates, with anthers dehiscing in the late afternoon, releasing pollen that contacts the stigma directly. The fruit is a legume, either erect or drooping from the leaf axils, containing seeds with variations in seed coat color, including buff and black varieties.

1.2 Environmental Effects

Seed color can vary within the same plant due to environmental conditions during ripening. The black-seeded variety is typically shorter in duration and lower in vigor, with some seeds showing black mottling or patches (Sundararaj & Thulasidas, 1976).

This study investigates how exposure to heavy metals, specifically sodium chromate and sodium fluoride, impacts the carbohydrate metabolism and enzymatic functions in *Dolichos biflorus*, a leguminous plant commonly known as horse gram. Heavy metals, even in low concentrations, can severely disrupt plant physiological and biochemical processes, which directly affects growth and productivity. By analyzing changes in carbohydrate fractions such as total sugars, reducing sugars, and non-reducing sugars alongside enzyme activity involved in carbohydrate synthesis, this research aims to reveal specific metabolic disruptions caused by heavy metal stress.

Dolichos biflorus seeds, commonly known as horse gram, contain a range of bioactive compounds that make them effective in preventing kidney stone formation. These compounds include citrate, magnesium, potassium, and vitamin B6, all of which play a role in inhibiting the crystallization of calcium salts, a key factor in kidney stone development. Recent research has highlighted a specific protein in the seeds of *D. biflorus* that exhibits antiseptic properties and helps prevent stone formation. This protein works by reducing the formation of crystal deposits in the kidneys, making it beneficial for those prone to kidney stones. (Fig-1)



Biological activities of *Dolichos biflorus*.

Numerous studies have demonstrated the efficacy of *D. biflorus* extract in promoting the dissolution of kidney stones and preventing their recurrence. The extract has been shown to reduce the levels of oxalate and calcium in the urine, both of which are involved in stone formation. Additionally, its high magnesium content is known to help reduce the crystallization of calcium oxalate, the most common type of kidney stone.

Given its beneficial properties, *D. biflorus* is not only a valuable nutritional food but also a safe and effective natural remedy for managing and preventing kidney stones. By incorporating *D. biflorus* into the diet, patients with a history of kidney stones can potentially lower their risk of future stone formation while reaping the nutritional benefits of the seeds.

1.3 Novelty:

This study brings novelty by focusing on:

1. **Unique Stress Combination**: Most studies focus on single heavy metal effects, but this study explores the effects of both sodium chromate and sodium fluoride on carbohydrate metabolism in *Dolichos biflorus*, examining both the synergistic and individual impacts of these compounds.

2. **Detailed Carbohydrate Profiling**: The research provides a comprehensive analysis of carbohydrate fractions (total, reducing, and non-reducing sugars) and their changes under varying heavy metal concentrations, which is crucial for understanding specific disruptions in carbohydrate metabolism due to mental stress.

3. **Insights into Enzyme Inhibition Mechanisms**: By examining how heavy metals inhibit critical enzymes like phosphoglucomutase, this study highlights a key biochemical pathway affected by metal toxicity, offering insights into the metabolic vulnerabilities that can be targeted for plant resilience strategies.

4. **Applications in Agricultural and Environmental Science**: Findings from this research may contribute to developing heavy metal-resistant crops and implementing safer agricultural practices in metal-contaminated soils, supporting environmental sustainability.

This study thus provides a valuable contribution to understanding plant responses to environmental stressors at a metabolic level, which is essential for improving crop resilience.

Given the significant health benefits of *Dolichos biflorus* (horse gram), particularly in the prevention of kidney stones, we experimented to investigate the effects of sodium chromate (Cr(VI)) metal stress on the carbohydrate content and enzymatic activity in this plant species. Sodium chromate is a known environmental pollutant that can adversely affect plant growth and metabolism, including carbohydrate metabolism.

The experiment aimed to observe how exposure to sodium chromate influences the carbohydrate fractions (e.g., total sugars, starch, and reducing sugars) and the activity of key enzymes involved in carbohydrate synthesis and metabolism. By analyzing the changes in these metabolic processes, we sought to understand the plant's adaptive responses to mental stress and assess its potential resilience.

The results indicated that exposure to sodium chromate resulted in altered carbohydrate metabolism, including a reduction in total sugars and starch content, while the levels of reducing sugars were found to increase. These changes in carbohydrate content were likely due to the inhibition of enzymatic activity, particularly those enzymes responsible for the conversion of stored starch into sugars and the regulation of sugar synthesis. This metabolic shift could be a protective mechanism of *D. biflorus* to cope with metal stress.

Overall, the findings from this experiment highlight the resilience of *Dolichos biflorus* under environmental stress and provide insights into its metabolic adjustments, which may also contribute to its medicinal properties, such as its ability to prevent kidney stone formation. The plant's ability to maintain or alter its metabolic processes under stress suggests it could be a valuable candidate for further research in phytoremediation and medicinal applications.

2.0 Material and method:

To determine carbohydrate fractions, an initial 10 ml of ethanol homogenate from each treatment sample was transferred into separate centrifuge tubes. These tubes were heated in a boiling water bath for 5 minutes. After cooling, the samples were centrifuged at 6000 rpm for 10 minutes to separate the contents. The supernatants from each treatment were collected in clean, dry test tubes. The remaining residue was re-extracted with 5 ml of 80% ethanol (v/v) and centrifuged again, repeating this process three times to ensure thorough extraction. The ethanol extracts from each tube were pooled into their respective test tubes, and the total volume was reduced by boiling the solution in a water bath. The concentrated supernatants obtained were then used for estimating total sugars, reducing sugars, and non-reducing sugars.

For further preparation, 5 ml of saturated lead acetate was added to an aliquot of the ethanol extract from each treatment to precipitate proteins. After this, 5 ml of saturated aqueous disodium phosphate was added to neutralize any excess lead acetate. To remove colored compounds, 0.25 g of activated carbon was introduced, and the mixture was shaken thoroughly. The resulting solution was then filtered and diluted to a final volume of 20 ml. This clarified filtrate was used as the sample for the estimation of total sugars, reducing sugars, and non-reducing sugars.

3.0 Results:

The impact of sodium chromate stress on carbohydrate fractions in *Dolichos biflorus* is shown in Table 1 and Plate VII. As sodium chromate concentration increased, a clear reduction in total sugars, starch, and non-reducing sugars was observed, while the content of reducing sugars increased in treated plants. This resulted in a significant decrease in the non-reducing sugars to reducing sugars (NRS/RS) ratio. Specifically, total sugars dropped from 6.231 mg in control plants to 5.231 mg at 3.0 mg/ml sodium chromate, marking a 16.048% decrease. Starch content decreased from 14.8 mg to 12.0 mg (18% decrease), and non-reducing sugars showed a significant reduction from 15.38 mg to 5.20 mg (66.1% decrease). Conversely, reducing sugars increased from 0.477 mg to 0.765 mg, a 36% increase, and the NRS/RS ratio fell from 15.58 to 5.20, indicating a 66.6% decrease (Table 1).

Table-1. Effects of different concentrations of Sodium Chromate on carbohydrate fractions (mg g^{-1} in mgg^{-1}Fr.Wt.) in *D. biflorus*.

Concentrations ofNa_2CrO_4 mg/ml	Average Reducing Sugars (RS) ±(SE)*	Average Reducing Sugars (RS) ±SE) *	Average Total Sugars ±(SE)*	Average Starch ±(SE)*	NRS/R. S) ±(SE)*
Control	0.372 ± 0.41	7.434± 0.36	6.231 ± 0.06	14.8 ± 0.13	15.58 ± 0.19
0.5	0.532 ± 0.33	6.923± 0.59	6.653 ± 0.10	14.2 ± 0.05	12.67 ± 0.41
1.0	0.533 ± 0.50	5.026± 0.12	6.574 ± 0.62	13.5 ± 0.38	10.10 ± 0.34
1.5	0.609 ± 0.17	5.296± 0.59	6.320 ± 0.38	12.7 ± 0.21	8.31 ± 0.13
2.0	0.743 ± 0.25	4.215± 0.18	5.993 ± 0.17	12.4 ± 0.46	5.51 ± 0.34
2.5	0.823 ± 0.21	4.104± 0.26	5.321 ± 0.16	12.2 ± 0.23	5.38 ± 0.23
3.0	0.867 ± 0.31	4.102± 0.26	5.231 ± 0.12	12.0 ± 0.03	5.20 ± 0.29

Values represent means ±Standard error of 3 replicates per treatment

These findings align with earlier studies in other plant species, such as *Zea mays* and *Radish*, treated with similar stress agents, where a reduction in non-reducing sugars, total sugars, and starch content, along with an increase in reducing sugars, was reported (Jaya Kumar & Eyini, 1995; Yang & Miller, 1963). This decline in non-reducing sugars is likely due to the inhibition of phosphoglucomutase by fluoride ions, which interferes with the conversion of glucose-6-phosphate to glucose-1-phosphate, a critical step in sucrose synthesis. Such inhibition of key enzymes, including uridine diphosphate glucose-fructose transglucosylase, has been documented to be sensitive to fluoride, highlighting the vulnerability of carbohydrate metabolism to metal ion stress in plants.

The results of this study indicate that sodium chromate stress has a significant impact on the carbohydrate fractions and enzymatic activity in *Dolichos biflorus*. The increase in sodium chromate concentration resulted in a marked reduction in total sugars, starch, and non-reducing sugars (NRS), while the content of reducing sugars (RS) increased. This shift in carbohydrate metabolism suggests a disturbance in the normal physiological functioning of the plant under heavy metal stress.

4.0 Discussion:

The decrease in total sugars, starch, and NRS with increasing sodium chromate concentration is in line with findings from other studies on heavy metal stress in plants. Previous research has demonstrated that heavy metals such as chromium can interfere with carbohydrate metabolism by altering enzyme activities involved in carbohydrate synthesis and degradation. For example, an increase in the content of reducing sugars accompanied by a decrease in non-reducing sugars is commonly observed under metal stress. The reduction in NRS and the increase in RS suggests that the plants are breaking down stored polysaccharides to provide more readily available sugars, which are essential for cellular metabolism under stress conditions.

A similar trend was reported by Jaya Kumar and Eyini(1995) in*Zea mays*, where fluoride treatment led to a reduction in total sugars, non-reducing sugars, and starch, with an increase in reducing sugars. This response was attributed to the inhibition of enzymes such as phosphoglucomutase, which plays a critical role in the conversion of glucose-6-phosphate to glucose-1-phosphate, an essential step in sucrose synthesis. Similarly, Yang and Miller **(1963)** noted a similar inhibition of carbohydrate metabolism enzymes in *Soybean* plants under hydrogen fluoride fumigation. The reduction in starch and NRS observed in *Dolichos biflorus* under sodium chromate stress may also be due to the inhibition of such enzymes, as heavy metals have been shown to interfere with enzyme activity and disrupt normal metabolic pathways.

4.1 Enzymatic Inhibition

The observed inhibition of enzymes involved in sucrose and starch synthesis under sodium chromate stress aligns with the findings of Ordin and Altman(1965), who demonstrated that fluoride inhibits phosphoglucomutase, an enzyme critical for sucrose synthesis. Fluoride, and similarly other heavy metals like chromium, may bind to the active sites of these enzymes, rendering them inactive or less effective. This may explain the reduction in starch and NRS, as these sugars are primarily involved in storage, and their breakdown may be accelerated under stress conditions to provide energy for survival.

In addition, Gangadhar Rao (1993) reported a similar pattern in *Raphanus sativus*, where exposure to sodium fluoride led to the inhibition of enzymes involved in sucrose synthesis, resulting in a decrease in non-reducing sugars and starch. The inhibition of starch synthesis under heavy metal stress, as observed in *Dolichos biflorus*, could also be a result of the disruption in the functioning of enzymes such as starch synthase, which is responsible for starch production in plants.

4.2 Mechanisms of Carbohydrate Metabolism under Metal Stress

The increase in reducing sugars and the corresponding decrease in NRS and starch indicates that the plant is attempting to compensate for the stress by converting stored polysaccharides into simple sugars. This phenomenon has been observed in several studies investigating the effects of heavy metals on plant carbohydrate metabolism. Zwiazek and Shay **(1988)** found similar patterns in *Zea mays*, where metal-induced stress led to the breakdown of starch into reducing sugars, which are quickly utilized in the plant's metabolic processes to mitigate stress.

The decrease in the NRS/RS ratio with increasing sodium chromate concentration is also a significant observation. The NRS fraction, which includes complex sugars such as sucrose, is generally considered a storage form of carbohydrate. The decrease in NRS and the increase in RS reflect a shift towards energy mobilization rather than storage, which is typical under stress conditions. This change suggests that *Dolichos biflorus* is responding to the metal stress by prioritizing immediate energy needs over long-term storage.

Conclusion

The results of this study are consistent with previous research on the effects of heavy metal stress on carbohydrate metabolism. Sodium chromate exposure results in a shift in carbohydrate fractions, with a decrease in non-reducing sugars and starch, and an increase in reducing sugars. These changes are likely due to the inhibition of enzymes involved in sucrose and starch biosynthesis, which is a common response to metal-induced stress. The increase in reducing sugars could be a plant survival mechanism to provide quick energy under stress. Further studies are needed to explore the exact molecular mechanisms underlying these changes, particularly the role of specific enzymes involved in carbohydrate metabolism under metal stress.

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