



The Canopy Advantage: Stomatal Feeding Maximising Pulse Production

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Physiological barriers that always affect pulse crops during the reproductive stage arise from fierce bioenergetic competition between the nitrogen-fixing root nodules and the floral sinks of the plant. Considering the fact that natural root and nodule activities are always reduced at the time of high nutrient requirement of pods, conventional soil-based fertilization practices will not help in keeping the plant alive and thus flower fall and low yield are common phenomena. This paper will focus on the practical importance and the exact physiological mechanism behind "stomatal feeding" where nutrients and PGRs are applied directly to the plant canopy through stomata. Instead of using the conventional route of fertilizer application to roots and subsequent shoot through soil biophysics, the stomatal feeding technique uses the pores on the leaves and leaf veins to supply necessary nutrients where they are metabolized. This targeted delivery of nutrients such as nitrogen, potassium, boron, and some specific PGRs can control the process of source-sink dynamics of the plant by postponing leaf senescence (source), improving pod retention (sink), and faster ATP-dependent phloem loading of carbohydrates. Fertilizer utilization efficiency is higher than 90% in stomatal feeding, which is an essential practice to improve yields by 12% to 25%.

Key words : stomatal feeding, foliar feeding, source-sink dynamics, phloem loading, soil biophysics

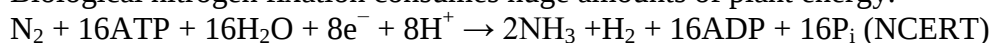
Introduction

Flowering of a pulse crop consumes metabolic energy from physiologically active tissues in the plant. In case of a short duration and indeterminate legume such as cowpea or green gram, there is a burden for internal mechanisms in the plant since it is required to produce new leaves as well as to provide support transition to flowering and seed filling process. As a result, this huge competition creates a 'source sink' interruption for translocating the essential nutrients which need to be absorbed by the root proteins and transported to the various tissues in the plant where it is utilised and metabolised, if this source-sink relation is disturbed it leads to imbalance in nutrient concentration in leaves which will directly impact with the amino acid and other protein synthesis in the plant. Whenever the roots fail to absorb and translocate sufficient amounts of nutrients from the soil and fulfill the requirements of canopy, the physiological stress created within the plant leads to flower abscission. Poor retention of reproductive organs in plants is the main cause of failure of pulse crops in realizing their potential yield. While the conventional basing of fertilization is essential and limits the nutrients available for the first bloom cycle, for nourishing the second and the third cycle of flowering, an adaptation in agricultural practices needs to be done. It is a scientifically proven

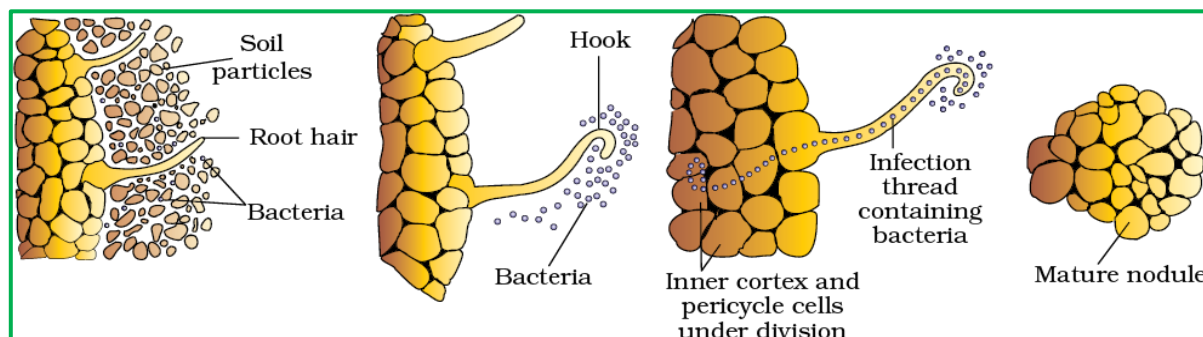
fact that the same elements absorbed through the root system of the plant can be absorbed efficiently from the foliage. By applying targeted macro and micro-nutrient and plant growth regulator spray on the crop canopy during the appropriate growth stage, "stomatal feeding" could be achieved. Stomatal feeding in this context means bypassing the root-shoot-leaf pathway which is associated with bioenergetics and directly feeding the leaves with essential nutrients through the stomata.

Physiological reasons for low yield in pulses

Diversion of bioenergy for protein synthesis: The ability to fix atmospheric nitrogen in their root nodules. This mechanism which operates below the soil operates not at zero cost. Biological nitrogen fixation consumes huge amounts of plant energy.



Symbiotic nitrogen fixation in pulses occurs due to a precisely regulated and coordinated physiological chain that includes complicated kinetics and huge bioenergetic costs. At first, infection kinetics depend on localized proliferation of *Rhizobium*, which adhere to root hairs, for this step to occur the plant has to sacrifice amino acids and sugars which are released from roots in order to attract the microbial members of the soil ecosystem causing bacteria to colonize around the root system leading to the curling and infection thread production that transfers bacteria to the root cortex and causes cell divisions to develop a fully-fledged nodule with vascular mass-transfer connection. Formation of nodule involves active division of cortical and pericycle cells which is stimulated by bacteria that entered into the system through the infection thread leading to the formation of nodule. In such a nodule, the catalytic reduction of nitrogen from atmosphere to ammonia occurs through the Mo-Fe protein nitrogenase enzyme, whose kinetics is extremely oxygen-sensitive. For this reason, leg-haemoglobin is produced in the nodule to oxidize oxygen and keep the strictly anaerobic conditions near the active center. From the bioenergetic point of view, the reduction of the nitrogen triple bond is very endergonic reaction that requires the consumption of 8 moles of ATP per each molecule of NH_3 . It means a huge metabolic cost of this reaction, which is provided by the respiratory system of the host plant which clearly indicates the diversion of energy that is obtained by the dehydrogenation and oxidation of photosynthetically fixed energy rich organic compounds to meet the energy requirement of the plant. After the ammonia is synthesized, the fast acid-base equilibrium at physiological pH makes ammonia protonated into very poisonous ammonium ions NH_4^+ . To prevent the poisoning, the plant needs to convert these ions into glutamic acid using its fast kinetics by the reaction with alpha-ketoglutaric acid and then obtain other essential amino acids via transaminations. And finally, the plant transforms these amino acids into amide structures like asparagine and glutamine or ureides with exceptionally high N:C ratio to achieve efficient mass transport in the xylem transpiration stream to the rest of the plant.



Competition for sugar and other compounds

Photosynthates are synthesized in the leaves of plants (mesophyll cells). These photosynthates then must reach the sites of new growth like developing flowers and fruits, or "sinks". This process takes place due to the fact that sugar is actively transported from the leaves to the phloem at high concentration creating pressure gradient and sucking up water

from the neighboring cells. This results in extremely high pressures and natural pumping of the sugary fluid throughout the plant through its veins. The flow of the sap takes place according to the pressure gradient and proceeds at a very high speed as the fluid moves from the high-pressure source in the leaves to the places of lowest pressure and unloading of the sugar. As soon as the plant begins flowering it hardly starts by opening all its flowers at once. The first flowers appear on the stem fast and develop at a high rate. They consume huge amount of sugars and thus create great drop of the pressure in the phloem at the site of their development. Being the first to act they create steep pressure gradient and become strong sinks. Now the mass flow of the plant is programmed in such a way that the plant receives a massive supply of sugars directly to the sinks created by the first flowers. As soon as the late flowers appear, they face great disadvantage as the first flowers have already taken over the pressure gradient and because the fluid is rushing towards the sinks the late appearing flowers receive decreased rate of photosynthetic influx. They do not have enough strength to generate enough force per unit area. Thus deprived of the sugars and structural carbon skeleton which is required to activate the floral physiology and the late formed flowers have physiological problems. They undergo miniaturization and grow small flowers and weak stems. Reproductive capacity is affected greatly as well as it leads to low pollen viability and decreased nectar production.

Slow dry matter accumulation

In general, pulses are known to have extremely slow growth, as the very first phase of the plant is characterized by very slow dry matter accumulation which represents an actual biological weakness of the crop. Such a phenomenon can be explained by the very nature of pulses, namely the low leaf area index (LAI) and low light extinction coefficient. Thus, there is the lack of "solar panels" with a very poor capability of interception and utilization of solar radiation. Moreover, pulse crops are usually characterized by intrinsically lower values of photosynthetic rate in comparison with cereals. Therefore, pulses cannot easily create the initial carbon reserves needed to ensure rapid vegetative development of the crop. However, this biological weakness is aggravated by the fact that these plants are usually cultivated on marginal lands and sown without proper land preparation and other early season agronomic practices by farmers who are often forced by the shortage of resources or other reasons to perform their farming operations very quickly. When the seed germination process occurs in improperly tiller, cloddy or compacted soil, the early root and shoot development is constrained by physical limitations which prevent from rapid increase in leaf area. In addition, the pulse crops are more and more often subject to extreme early-season weather conditions such as heat waves and soil moisture deficit occurring at the moment of vulnerability of the plants. As a result of interaction of these adverse weather factors and poor management, there appear extremely harmful physiological effects at cellular level of plants. The first thing to do with the stressed plants is to attack the photosynthesis. The core of the photosynthesis is the Calvin cycle which is responsible for transformation of carbon dioxide into sugars. Under the influence of extreme temperature and drought, the stress conditions lead to ROS accumulation and thermal destruction of protein catalysts of this cycle. More precisely, under the described conditions the enzymes RuBisCO and RuBisCO activase which play the key role in photosynthesis become degraded. The absence of necessary catalysts results in the inhibition of dark photosynthesis. Therefore, the pulse plant with tiny leaf area and grown in the improperly prepared soil loses one of the most important features of its existence – the capability to produce food.

Reduction in nodule activity: In a pulse crop, the roots and the symbiotic bacteria work as a team to set up a thriving nitrogen-producing system. The sugars which are made in the leaves of the pulse crop are passed on to the root nodules. The root nodules then use these sugars to fix nitrogen and turn it into a form that the crop can make use of. This process is known as amination. The bacteria take the ammonia that they have fixed and combine it with carbon to produce amino acids such as glutamate. As a result, the root nodules of the pulse crop become larger and heavier, giving the crop a supply of amino nitrogen which in turn helps it

to grow and expand its leaves. All the changes take place as soon as the pulse crop begins to flower. From that point on, the crop directs its energy towards the developing pods in the roots. The sugars which previously reached the roots are now being sent to the seeds. Since the root nodules no longer receive the carbon they require, they start to break down. In 1988 Sinha and his colleagues discovered that when the root nodules collapse the pulse crop's ability to produce nitrogen drops suddenly. This is a problem since the developing seeds need a lot of nitrogen in order to form protein. The underground nitrogen-producing facilities of the pulse crop come to a stop and the crop has to find some other means of obtaining nitrogen. To obtain the nitrogen it needs, the pulse crop begins to break down its leaves. Since it cannot get the nitrogen from the root nodules, it uses the nitrogen which it already has in its leaves. It breaks down its proteins, among these being the ones that enable it to make food from sunlight. The amino groups from these proteins are taken and attached to new carbon skeletons in order to form new amino acids, after which the amino acids are sent to the developing pods. This ensures that the seeds receive the protein they require. It has a negative effect on the yield of the pulse crops. The leaves lose the ability to use nitrogen and to produce food from sunlight. The enzymes which help the pulse crop to make food are broken down and sent away. As a result, the leaves turn yellow and die prematurely. The pulse crop is reducing its productivity by using its leaves to fill its pods. That is the reason it is difficult to keep the leaves in a healthy condition during the season when the pulse crops are being grown.

Translocation Difficulties and Pressure Gradients: Failure to partition the assimilates effectively is primarily a translocation problem regulated by the Münch pressure-flow model.

Sink Strength and Phloem Unloading: Translocation is based on a hydrostatic pressure gradient existing between the "source" (mature photosynthesizing leaves) and "sink" (growing parts). The active vegetative apex is an extremely strong sink—unloading of sucrose from phloem sap takes place there rapidly, creating a steep local pressure gradient attracting sap upwards.

Reproductive Weakness in Drawing Capacity: Flowers and young pods constitute a relatively weak sink—its drawing capacity is relatively low. In the presence of an active vegetative apex drawing assimilates through a steep gradient, flowers fail to create a competing pressure differential in the phloem pathway, being consequently bypassed by the massive flux of carbon and nitrogen.

Erratic Summer Rainfall Effect: Phloem transport is dependent entirely on turgor pressure created by water inflow into the source leaves via osmosis. Erratic summer rainfall conditions causing water stress result in a reduction of water potential and, hence, in a decrease of initial turgor pressure in the source.

System Failure: Under such circumstances mass flow decreases throughout the plant. The vegetative apex uses its dominant position and all remaining flow, while the reproductive sink becomes totally deprived of assimilates. This leads to failed pod set, seed filling, and poor partitioning coefficients.

Carboxylation mechanism: C3 pulses carbon assimilation are taken care of an enzyme named RUBISCO, which is dual in nature. It is sensitive to oxygenated conditions which denotes that it will react with oxygen instead of carbon dioxide, diverting the biosynthetic pathway to photorespiration, which will consume plant's metabolic energy. Assured carboxylation is absent in C3 pulses due to nature of RUBISCO.

Stomatal feeding/Foliar nutrition-The solution : This method uses the openings on the leaves called stomata and the outer layer of the plant to get nutrients into the plant quickly. By changing how the plant absorbs nutrients from the roots to the leaves stomatal feeding has important benefits for farming.

Rapid Stress Mitigation: This method gives the plant a response. It is the way to help plants when they are under stress like during a drought or when they are sick or attacked by insects. The roots might not work well during these times.

Overcoming Soil Limitations: When the roots cannot take in nutrients because the soil is too dry too cold or has too much water or when nutrients get stuck in the soil stomatal feeding helps the plant get what it needs. This is important, during the times when the plant is growing the most.

Morphology: Pulses with trifoliolate leaf like the mungbean and urdbean, or pinnately compound like the chickpea and lentil, have leaves of divided form. Such leaves have two advantages over simple ones. They offer a larger boundary layer, and hence, a greater surface/volume ratio to the leaf canopy, and they create a microclimate of high humidity around the canopy. A foliar spray (2 per cent DAP or micronutrient solution) applied during the active vegetative phase will have an extensive catch area. In addition, the microclimate will prolong the time for which the droplets will remain in a liquid form, thus giving them sufficient time to penetrate the cuticle and/or the stomata of the leaflets.

Venation: The dicot nature of the pulse crops comes into play here, for they offer a rapid transit system through their reticulate venation. The transport system offered by reticulate venation is of immense value in the case of foliar feeding. It ensures rapid loading of the ions caught in the leaf mesophyll into the vascular bundles so that they may be quickly transported to the various target sites within the plant body. As the pulse crop progresses into the pre-flowering stage from the vegetative growth phase, its requirements for nutrients increase manifold in order to meet the demands of reproductive growth. During this period, the root system may not be able to keep pace with the demand, especially since there is a natural tendency for the root activity to slow down. In such a situation, the reticulate venation acts as a rapid transit system, getting the ions from the leaf (where they were caught during foliar feeding) to the target sinks (the floral buds) in good time. With these advantages of morphology and anatomy in mind, it becomes apparent why foliar feeding, especially at pre-flowering, is such a valuable tool in the management of pulse crops.

Escaping the Soil Trap: Understanding why Foliar Nutrition is a Good Idea for Pulses

To understand the value of foliar nutrition in pulses we have to start by looking underground – literally. There are serious nutritio-physiological reasons why relying solely on soil-applied fertilizers during critical growth stages is not a good idea. When the matric potential of the soil is high (i.e. dry), applying heavy rates of granular fertilizer on top of the soil increases the osmotic potential of the soil solution. This can trigger significant osmotic stress on the root system which then has to invest a lot of energy to absorb water from this hypertonic solution. On the other hand, soil biophysics can impose serious constraints on the availability of fertilizer nutrients. For example P is fixed rapidly by the colloids, N is lost through leaching and volatilization and root respiration is inhibited when the soil becomes dry, restricting the assimilation of nutrients during the crucial pre-flowering stage. By comparison, foliar application delivers the nutrients directly to the canopy, thus circumventing the hostile environment of the soil matrix.

Foliar Uptake Mechanisms: Going from the Surface to the Vascular Bundles

The leaf surface has two main barriers, namely the cuticular wax and trichomes, which are hydrophobic. High quality agrochemical surfactants (wetting agents) are often needed to reduce the surface tension of the nutrient solution so that it can penetrate the leaf surface via the stoma or cuticular pockets. Once inside the cell, nutrients can follow two main pathways: (apoplast-intercellular and symplast-intracellular). The latter enables faster foliar absorption. It involves transport of ions and molecules from cell to cell across the plasma membrane via plasmodesmata. The short distance transport ends in the vascular bundles of the leaf. From there, the long distance transport begins via the phloem to supply the various ‘sinks’ in the plant such as root nodules, leaves, and flowers.

Formulating Nutrient Solutions for Foliar Applications in Pulses

Since leaves have adapted to capture light, foliar products have to meet certain physiological and molecular requirements in terms of solubility, purity, and salt index to avoid leaf burn. In terms of N nutrition, urea is the preferred source since it is a neutral molecule that can penetrate the cuticular wax with ease. However, urea has to have low levels of biuret (a toxic byproduct) to prevent leaf scorch. Urea-based foliar sprays are very effective in boosting the N nutrition status of the plant during flowering. A 2% solution is sufficient to rescue a pulse crop from total N deficiency during the flowering stage. Since the foliar-applied nutrients are delivered directly to the site of assimilation, their mobility status within the phloem determines how soon they can be utilized by the plant. For example N, P, and K are mobile nutrients, which means that they can be translocated from one part to another. On the other hand, Ca and most micronutrients including Fe and Mo are considered immobile or weakly mobile and it can take up to 14 days before their deficiency symptoms become visible. This information is very important, especially when planning foliar interventions for P, K, S, Mg, Mn, Zn, Cu, and Cl.

Hormonal Regulation of Source-Sink Relationships in Pulse Crops

Modern agro-science has demonstrated that crop productivity can be manipulated to a greater extent by the regulated use of Plant Growth Regulators (PGRs). By exogenously-applied plant hormones we can influence the physiology and morphology of the crop to frame the source-sink relationships. For a pulse crop, building a greater 'source' capacity (greater leaf area) and stronger 'sink' (flower and pod development) is critical for maximizing yield.

Source Strength: Auxins (IAA, NAA) and Brassinolides are growth hormones that increase vegetative growth and LAI when applied during the vegetative stage. A greater number of pods and greater LAI translates into higher crop growth rate (CGR) due to higher assimilation of CO₂ and photo-assimilate production.

Stimulating Development of Greater Sink Strength: The main objective during reproductive stage is to ensure that the source assimilates are directed into the developing pods and not into the vegetative parts of the plant. This can be achieved by using PGRs such as GA3 or specific Brassinosteroids (BRs) to encourage pod set and increase number of pods per plant. This results in increased seed weight and higher harvest index as well as higher yield.

Importance of surfactants: The chemical complexity of the cuticular leaf that is designed to oppose the entry of water reveals the importance of surfactants in effective foliar nutrition. Surfactants reduce the surface tension that reduces the angle of contact so that drop spreads and maximizing the epidermal surface area in contact with nutrient ions.

Factors that influence foliar nutrition

- ❖ **Water Potential and Solute Concentration Spray Concentration Gradient:** The spray droplet has a very negative solute potential due to the presence of salts. In order for the plant to absorb the nutrients while remaining in an aqueous solution without losing any cellular water, the relative humidity of the environment needs to be such that the solute potential of the spray droplet does not decrease excessively. In the case where the concentration of the spray is excessive (hypertonic spray), it reverses the water potential gradient and draws water out of the epidermal cells leading to plasmolysis and "leaf burn".
- ❖ **Cuticular Hydration:** Increased relative humidity causes an increase in the water potential of the air, thereby hydration of the cuticular waxes resulting in larger aqueous pores, thus making the absorption of solutes via diffusion along their concentration gradient possible.
- ❖ **Vacuolar Osmoregulation:** The ions once they cross the plasma membrane into the cytosol, immediately reduce the solute potential in the cytosol. In order to prevent the buildup of ions in the cytosol as well as ensuring metabolic processes continue normally, the cell actively transports these nutrients into the vacuoles.

- ❖ **Turgor Pressure Maintenance:** By doing so, the solute potential within the vacuoles decreases and attracts water into the cell, thus maintaining positive turgor pressure.
 - ❖ **Translocation & Source-Sink Relationship Phloem Loading:** The loading process into the leaf (the source) is only the beginning. In the case of mobile nutrients, like Nitrogen, Phosphorus, and Potassium, these are actively loaded against their concentration gradient into the phloem companion cells, a process that uses ATP (energy)
 - ❖ **Mass Flow Creation:** Active loading of these nutrients into the sieve tubes greatly reduces the solute potential of the phloem, which then draws water from the adjacent xylem resulting in high hydrostatic pressure.
 - ❖ **Pressure-Flow to Sinks:** This creates high pressure in the phloem and creates the mass flow of the nutrient rich sap towards the sinks (roots, developing grains or newly developed meristematic tissue). These sinks have reduced pressures as a result of phloem unloading.
- Immobile Nutrients:** Calcium and Boron ions do not move much in the phloem as they are bound specifically to the cell walls and are not actively loaded into the symplast. Foliar spray of these elements addresses deficiency locally and has almost no use of systematic translocation of N-P-K.

Nutrients and Plant Growth Regulators (PGRs) application to the leaves in pulses directly interferes with the source-sink balance, helping the plant to cope with its most difficult physiological stage - the reproduction phase and pod filling. Flowering and pod development start in pulses with the decrease in root activity and nodulation senescence. Just when the need for carbon and nitrogen reaches the peak, their uptake from the soil declines. Foliar fertilization fills the gap by directly activating the photosynthesis (the "source") and increasing the allocation of the assimilates to the developing pods (the "sink").

Maintaining the Source Activity ("Stay-Green Effect")
Maintenance of Photosynthesis: Foliar nitrogen (urea) and magnesium help in maintaining chlorophyll and RuBisCO enzyme activity. Leaf senescence delays, extending the functional life span of the "source" and thus providing a continuous flow of photosynthates.

PGRs Synergy: Cytokinins and some brassinosteroids act in the foliar formulation by reversing senescence-related gene expression. The "photosynthesis factory" stays alive longer during the late pod-filling stage.

Strengthening Sink Strength and Preventing Pod Abscission.
Abscission Prevention: Pulses experience extremely high flower and pod abortion rate due to hormonal imbalance in the plant favoring abscission (relatively high levels of ethylene and abscisic acid in comparison with auxins). Auxins applied to the leaves (e.g., NAA) reduce abscission rates by strengthening the abscission zone.

Increasing the Sink Need: Pod that remains on the plant is an active sink for the carbohydrates. PGRs stimulate cell division and growth of the ovules increasing the metabolism intensity. Higher metabolism means more steep concentration gradient, and therefore a better "pulling" of the carbohydrates from the leaves.

Carbohydrate Translocation (Phloem Loading)
Osmotic Agents: Potassium (K) and Boron (B) are essential for carbohydrate transport. Foliar potassium serves as an osmotic agent during the phloem loading. It stimulates proton ATPase which actively loads sucrose into the sieve-tube elements resulting in the generation of hydrostatic pressure for the mass flow to the pods.

Boron Effect: Boron accelerates carbohydrates transport through the cell membrane by the formation of ionizable sugar-borate complexes. Carbohydrates move faster from the actively metabolizing leaf cells into the vascular system.

Conclusion By keeping the source green and active, preventing sink from abortion, and stimulating phloem loading, the plant distributes a higher portion of its biomass into the seeds. The increase in harvest index, test weight and yield is achieved.

Conclusion

Leveraging the plant canopy as an immediate source for nutrients completely revolutionizes the way pulse farming is conducted. The practice of harnessing stomata and cuticle routes for

feeding leaves enables the farmer to circumvent unpredictable soil limitations and natural reduction in root activity, thus providing nutrients to the metabolizing parts at the very time when the requirement for sink becomes maximum. The “canopy advantage” leads to efficient fertilizer utilization above 90%, resulting in a 12% to 25% crop yield improvement through ensuring vegetative growth, avoiding flower abortion, and optimizing pod formation in the late stages. Moreover, stomata feeding acts as an important safeguard for farming in marginal lands and is economically beneficial since it allows for the combination of nutrients with the agrochemicals in one pass. Although the deficiency of technical know-how continues to be a barrier for adoption of this technology in India, moving our agricultural efforts upwards towards leaves is absolutely necessary. The physiological route must now be exploited not as a complementing technology but as an exact science of overcoming biological barriers and optimizing the pulse farming process.

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