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Millets of Cold Semi-Arid Regions: Vital Facts in Starch Content and Composition

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ABSTRACT

Nutritious grains such as millets, which are popular as candidates for diet diversification, have an inherent capability to thrive under adverse growth conditions of temperature. This review draws attention to the effect of cold temperature on macronutrients such as starch and amylose, in millets and related cereal grains. It focuses on the changes in starch biosynthesis mechanisms and the resulting influence on nutritional properties. It also describes how cold temperature is beneficial in increasing the concentration of amylose within starch granules that leads to health benefits for patients suffering from type 2 diabetes and cardiovascular ailments. Various studies on millets growing in hot semi-arid regions have been carried out, although, with a primary focus on their proximate composition and nutritional properties only. In this review, special attention has been drawn to the scarcely explored area of the effect of cold temperature on the growth of millets in cold semi-arid regions. With evidence to support the effect of cold temperature in increasing amylose content within starch granules, it is imperative to study millets thriving in high altitude semi-arid regions which exhibit very cold temperatures during growth (10-15°C). We have also proposed studying the effect of cold temperature on starch biosynthesis in correlation to phytohormonal regulation of starch biosynthesis. Phytohormones are themselves controlled by temperature variations and may act upstream of starch biosynthesis to alter the accumulation of starch components such as amylose. Millet populations growing in cold semi-arid regions are potential candidates for revealing genetic diversity that exhibits higher amylose content.

Key Words: Millets, Semi-Arid, Starch, Amylose, Cold Temperature, Phytohormones

INTRODUCTION

The world's dietary dependency on a mere 1% of the 250,000 consumable plant resources[1], holds a clue for alleviating food and nutritional insecurity slowly making inroads in recent times. In the wake of Climate Change (CC) and soil erosion, the prevailing monocultural systems of agriculture have put food resources at risk[2], and the need for conservation and sustainable use of crop diversity is all the more prominent.[3] Diets dependent on a single cereal grain tend to be deficient in essential nutrients. It is imperative to diversify our cereal sources in the diet, to achieve both food and nutritional security.

Millets are climate compliant staple grains that promise nutritional security.[4] They are ancient grains originating from Africa and Eurasia, mainly grown in the dry semi-arid (hot and cold) regions of the developing world, and contributes to 97% of the world's total millet production.[5] They are cultivated in hot as well as cold semi-arid regions, such as in the hilly tracts of the Himalayan ranges.[6] Millet species are primarily divided into two categories, the major millets comprising of Pearl millet (*Pennisetumglaucum*) and Sorghum (*Sorghum bicolor*), and the minor millets comprising of Foxtail millet (*Setariaitalica*), Proso millet (*Panicummiliaceum*), Finger millet (*Eleusinecoracana*), Kodo millet (*Paspalumscrobiculatum*), Barnyard millet (*Echinochloafrumentacea*) and Little millet (*Panicumsumatrense*).[7] Pearl millet and Foxtail millet is the most popularly cultivated species, with Pearl millet occupying 95% of the world's total millet production. Finger millet, grown in East Africa and Southern India, along with the other millet species is primarily region-specific. While Kodo millet is confined to the tropical regions of Africa, Barnyard millet, the fastest growing millet (6 weeks), along with Little millet is mainly grown in south-east Asia.[5,8]

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NUTRITIONAL PROFILE OF MILLETS

The millet grain is essentially made up of macronutrients, such as carbohydrates and proteins, as well as, micronutrients such as dietary fibre, proteins, fat, and phytochemicals. An average value of 7-12% protein, 15-20% dietary fibre, 65-75% carbohydrates and 2-5% fat, makes them nutraceuticals that provide a balanced source of energy and nutrition, especially in populations with special dietary requirements, such as for coeliac and diabetic patients.[8] The different millet species are highly variable in their nutrient profile, with pearl millet containing higher protein (12-15%), finger millet containing higher calcium (300-350 mg/100 g) and lower fat percentages (2-5%), and the small millets rich in phosphorous and iron.[8,9] The proteins of millet grains provide an excellent amino acids profile as well, as compared to other major cereal grains such as maize. Pearl millet is rich in niacin, and finger millet in sulphur-containing amino acids.[8]In their whole-grain form of consumption, the dietary fibre, phenolic compounds and micronutrients present in the seed coat also contribute to their superior nutritional value.[10]

MILLET STARCH AND ITS COMPOSITION

Starch is the major macronutrient present in millet grains, making up about 65-75% of its grain weight, with a large proportion of this polysaccharide organized in millets as non-starchy dietary fibre which imparts a low glycemic index property to the grain.[8] Millet grain carbohydrates accumulate as discrete water-insoluble granules of varying shapes and sizes that are species-specific.[11] Grain endosperm is known to contain starch granules of three possibly different morphologies, the A, B and C-type of starch granules.[12] The different types of granules vary considerably in their physiochemical properties, which depends on the type of glycosidic linkage that makes up the polymers within the granule.[12] Starch granules are made up of two types of anhydro-glucose biomacromolecules, amylose, a predominantly linear glucan polymer with very few branches, and amylopectin, a highly branched polymer, along with minor quantities of protein within the granule.[13] The synthesis of these amylose and amylopectin chains in starch granules of cereals, involves four classes of enzymes that utilize the nucleotide sugars translocated from leaves to storage organs, to synthesize starch within amyloplasts.[14] Synthesis is initiated by the first class of enzymes, Adenosine Diphosphate (ADP)-Glucose Pyrophosphorylase (AGPase), which catalyzes the production of the glucose donor, Adenosine DiphosphateGlucose Pyrophosphatase (ADPG), from Adenosine triphosphate (ATP) and glucose-1-phosphate, already present in the amyloplasts. The ADP-Glucose produced then donates glucose units for elongation of amylose chains and amylopectin main chains, catalyzed by Starch Synthases (SSs), which are the second class of enzymes which extend the growing amylose and amylopectin main chains by addition of glucose units using α-1,4-linkages.[14] Starch Synthases (SS) are of two types, soluble and granule bound. The Granule Bound Starch Synthase (GBSS), also known as the Waxy protein, is the only enzyme solely committed to synthesizing the amylose component of starch using α-1,4-linkages.[15] The Starch Branching Enzymes (SBEs) introduce branch points in the growing amylopectin main chain and link it with a pre-synthesized glucan chain using α-1,6-linkages. The Starch Debranching Enzyme (DBE) trims the irregular amylopectin structure to produce an orderly branched amylopectin polymer.[14] In cereal endosperm, the dry weight of starch within a granule is typically made up of 30% amylose and 70% amylopectin polymers.[11]

Variation in Amylose Content within the Starch Granule

The accumulation of amylose polymers in starch granules may vary according to the plant species, variety, plant organ, developmental stage of the organ, and the conditions of plant growth.[16] Variation in the composition and ratio of amylose to amylopectin within the starch granule determines the area of applicability of the cereal grain. Amylose free (completely waxy) starch has uses in the adhesive, textile and corrugating industries, whereas starches with higher percentages of amylose (>40%) are used in the paper and pulp industry (gums & candies). Chemical modifications made to waxy starch have further diversified its application in the food industry.[11] In previous studies, partially waxy (0-25% amylose) maize and wheat were used for the production of certain types of Asian noodles, e.g. Japanese noodles.[17] Such modifications have opened new avenues for regulating the starch composition and incorporating uncommon grain into the daily diet like processed foods such as cakes/tortillas, noodles and pasta.[18]

In recent years, considerable research has been focused on exploring and designing high-amylose starches for their significant role in maintaining good health.[19]An increase in amylose concentration reduces starch digestibility, rendering it resistant to digestion. This leads to lower postprandial insulin response and low glycemic index owing to increased enzyme resistance.[20] On the basis of this digestibility of starch, it can be divided into available and unavailable carbohydrates. Unavailable carbohydrates or resistant starch is highly desirable for people suffering from obesity and associated diseases such as Type 2 diabetes and cardiovascular ailments.[21]

The predominant cause of an increased amylose concentration is attributed to its regulation by Granule Bound Starch Synthase1 (GBSS1). The level of expression of *GBSS1* is directly proportional to amylose accumulation.[22] Reduction in the accumulation of amylose is a result of allelic variations in the *GBSS1* gene that leads to its altered level of expression.[23]

The millet waxy gene contains 14 exons and 13 introns.[24]In foxtail millet, molecular analysis of landraces has identified the presence of transposable elements [transcriptionally silent information (TSI)-2 and TSI-7] in intron 1 or exon 3. These landraces with insertions of transposable elements were found to originate from geographically distinct regions[25], thus indicating the presence of genetic diversity among landraces separated by geographical boundaries. In a study conducted for 113 foxtail millet accessions, molecular differences in the waxy gene were found to be due to single nucleotide polymorphisms. Studies on foxtail millet varieties have also identified insertions of multiple transposable elements into the waxy gene that alter the level of protein expressed.[23,25] In a foxtail millet waxy landrace, Shi-Li-Xiang (SLX), re-sequencing identified SNPs that were then used as markers to sequence the *GBSS1* gene and reveal insertions of transposable elements in the waxy allele.[26] These studies indicate the presence of genetic polymorphisms in landraces growing in isolated geographical locations with adverse environmental conditions.

THE EFFECT OF TEMPERATURE ON STARCH AND AMYLOSE OF CEREAL GRAINS

Growth and metabolism in plants are greatly affected by environmental conditions. In addition to the effect of breeding practices or genotype differences, the nutritional content of the grain is also influenced by environmental factors, such as season, temperature, rainfall distribution and growth location. These factors are major determinants of grain structure and composition, which in turn influences properties such as starch functionality.[27,28] Environmental factors are known to affect starch biosynthesis, composition and Physico-chemical properties.[29] Changes in environmental conditions, which in many cases may be adverse or sub-optimal, affects the accumulation of starch polymers and changes the biochemical composition of the grain, which in turn alters its resulting nutritional potential.[29] Studies on growth temperatures affecting grain development have revealed that it alters starch content and composition, including amylose content, amylopectin chain length distribution and the number and morphology of starch granules. One way in which temperature stress regulates plant growth is by altering either the expression level or the structure and activity of enzymes.[29]

The effect of cold temperature on cereal starch biosynthesis is not as disruptive as high temperature. While on one hand, cold temperature results in poor physiology of plants, such as leaf yellowing and withering, on the other hand, it is known to enhance the level of certain nutrients within the cereal grain. Crops are grown at higher altitudes, experiencing cold temperatures have been found to have higher starch content.[30] Cold favours a prolonged duration of starch accumulation or slower grain filling, culminating in a greater than 50% increase in yields of the grain starch as compared to grains developed at warmer climatic conditions. Starch accumulation on the other hand is slower when high temperatures act at the grain filling stage.[31,32] Cooler days and cooler nights accelerate starch accumulation in developing grains as compared to hot days and cool nights, or hot days and hot nights, which tend to slow down the window of the starch accumulation process significantly. A peak in the transcript level of starch biosynthesis enzymes Adenosine diphosphate (ADP)-Glucose Pyrophosphorylase Small Subunit (AGPS) gene, Starch Synthase (SS) I to Starch Synthase III, Starch Branching Enzymes (SBE) I and II, and Granule Bound Starch Synthase (GBSS) has been observed to immediately precede this increase in starch accumulation.[33, 34]

In addition to the effect on starch biosynthesis and accumulation, cold temperature also seems to influence the structure and composition of the starch product within the grain. Starches developed at cold temperature contain a greater proportion of B-type starch granules, while hot temperatures during the day and night favour the predominance of A-type starch granules.[35] A-type starch granules contain a 6-9% increase in amylose content as compared to B-type granules. Based on the studies carried out on the effect of the size of the starch granule on starch quality and functionality, starches grown at colder temperatures exhibiting a higher proportion of B-type starch granules would have higher water absorption capacity[35], owing to their larger surface area compared to volume, which also creates a more unstable crystalline structure within these granules. These starches, therefore, have more firmness and less stickiness in their cooked form, which is an indication of improved starch quality.[36] The quality or palatability of the cereal grain also improves at close to 20°C, due to better grain filling which is disrupted at temperatures higher than that.[37]

Low temperature or cold seasons also tend to increase the amylose/amylopectin ratio within starch granules, while high temperatures decrease the amylose content.[38,39]In certain cases this increase or decrease is variety dependent. Although there have been fewer studies on cold temperature affecting starch functionality, it has been established that cold temperature promotes the accumulation of amylose polymers.[40] Higher amylose content has been observed in cultivars when they are grown either during the coolest seasons of the year or at cooler locations.[28,41,38] Extensive studies on the effect of cold temperature on millet starch are lacking, nevertheless, studies on wheat[42], rice[43,32] and maize[44] have been carried out to reveal that the increase in amylose as observed at lower temperatures is due to enhanced GBSS1 enzyme activity.[29] The amylose content in wheat and rice was found to be higher due to an increased GBSS activity, which was higher in rice grains grown at 15°C compared to 25°C.[32] Similar increase in amylose content was reported in maize crops that experienced a higher number of colder days during growth.[44] This marked increase in GBSS enzyme activity at lower temperatures is in contrast to other starch biosynthesis enzymes, which display a lower level of activity at low temperatures, indicating the significant role of Granule Bound Starch Synthase in increasing amylose concentration during growth at lower temperatures.[43,32]

This temperature-dependent regulation of GBSS1 activity is post-transcriptional.[45]Efficient splicing in the leader sequence of the 5' intron of GBSS1 is required for proper levels of amylose accumulation. Splicing of the GBSS1 intron is more efficient in the genotypes containing the GT-Single Nucleotide Polymorphism than in the genotypes containing the TT-SNP in the leader sequence leading to high amylose and low amylose phenotypes respectively.[45] Lower temperatures between 10-18°C increase the efficiency of splicing for the pre-mRNA of GBSS1, which leads to a higher accumulation of its transcript and protein levels.[46]

On the other hand, heat stress acts on starch biosynthesis to reduce overall starch content by reducing the activity of SSs and AGPases in temperate cereal grains such as wheat[47], at temperatures above 35°C, whereas it negatively impacts the activity of SBEII in tropical cereal grains such as maize and rice, at temperatures exceeding 25°C.[48,49] Starch composition is also altered at temperatures above 30°C, which reduces amylose content by 20% in maize and rice, but increases it in wheat by accelerating SS activity.[33] A similar tend with amylopectin chain-length distribution was also observed in rice which had longer glucan chains[38], in contrast to shorter chains of glucan observed in wheat at temperatures above 30°C.[50]Heat is also known to affect shape, size, structure and fissuring of the starch granule. For example, the hot temperature during growth was found to cause a reduction in the size of starch granules for wheat and barley but an increase in the proportion of A-type starch granules.[29,33]

THE INFLUENCE OF PLANT HORMONES ON STARCH CONTENT

The content of starch in cereal endosperm is influenced either directly or indirectly by factors other than temperature alone. Plant hormones, soil and nutrition, as well as, other growth factors such as light and CO_2 levels have major implications on starch accumulation.[51-53] Among these, starch accumulation in amyloplasts of storage organs is majorly controlled by plant hormones that act either synergistically or antagonistically to control the molecular mechanisms of nutrient biosynthesis.[54] Some studies have verified the effect of phytohormones such as Abscisic acid (ABA), Cytokinins, Gibberellins, Auxin and Brassionsteroids on starch accumulation, and have found ABA to help increase starch accumulation by more than three folds. Yet again, this stimulus works by enhancing the expression levels and activity of crucial starch biosynthesis enzymes, SpAPL2 & SpAPL3 (components of the ADP-Glucose Pyrophosphorylase (AGPase) starch biosynthesis enzyme) in this case.[55] Auxin, on the other hand, represses the AGPase enzyme activity when acting at low concentrations, unlike cytokinin which stimulates it.[56] During the early stages of brain development, cytokinin regulates starch accumulation by altering the design of the grain filling model and affecting the final percentage of starch within the grain.[57]

Plant hormones are indirectly regulated by the environment to control starch biosynthesis and accumulation. Since the environment plays a crucial role in directing the regulation of hormonal levels in plants, it is important to analyze the role of climatic conditions while studying the relationship between phytohormones and starch accumulation.[44] Water deficit, in general, has been seen to reduce starch content in wheat by reducing the activity of its biosynthetic enzymes, SSs, AGPases and GBSS. [58] Auxin has been found to inhibit, and cytokinin to enhance the transcript levels of starch biosynthesis enzymes such as GBSS, SBE (Starch Branching Enzyme) and ADP-glucose pyrophosphorylase small subunit gene (*AGPS*).[59] While these phytohormones affect the transcript levels of the enzymes responsible for starch biosynthesis, lower temperatures control phytohormone levels [60], to consequently influence downstream starch accumulation. For example, root cooling due to cold exposure has been found to decrease the endogenous levels of cytokinin in shoots and increase the levels of auxin (Indole-3-acetic acid)[60], thus indicating a decrease in starch accumulation (Figure 1). This is mediated by inhibition or stimulation of starch biosynthesis enzymes including GBSS1, SBE and AGPS. Various such studies on cereal grains indicate that millet populations growing in cold semi-arid regions may have a different interplay of phytohormones and starch biosynthesis to alter starch and amylose content in a unique manner, when compared to growth at normal temperatures.

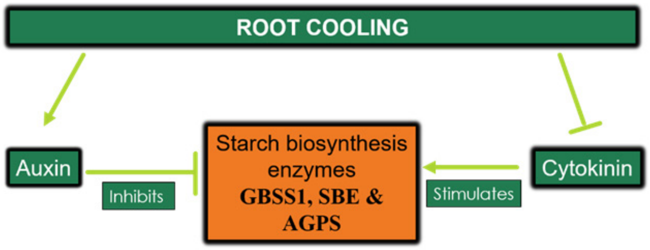

Figure 1: The Effect of Cold Temperature on Phytohormones that Regulate Starch Biosynthesis. GBSS1- Granule Bound Starch Synthase 1; SBE- Starch Branching Enzyme; AGPS- ADP-Glucose Pyrophosphorylase.

Moreover, the role of phytohormones in regulating starch content and biosynthesis in the cereal endosperm under conditions of lower temperature and water deficit has not been extensively explored. There is some evidence to indicate that an Abscisic Acid (ABA) mediated stress-signalling pathway is responsible for reducing the activity of starch biosynthetic enzymes such as SSs and SBEs during drought stress, however, GBSS activity is not affected in this ABA-dependent manner during drought stress.[28] This indicates that GBSS activity may be influenced by some other upstream acting phytohormone to regulate its activity during cold or drought stress.

This evidence indicates that crops growing at adverse conditions of temperature may exhibit differences in the content and composition of plant products due to up-regulation or down-regulation of plant hormones.

CONCLUSION

Nutritionally superior grains such as millets are also climate-compliant crops capable of withstanding adverse environmental conditions, including cold temperature. Cold temperature has a significant impact on grain properties such as starch content and composition. Moreover, cold exposure leads to fluctuations in the concentration of plant hormones that regulate starch biosynthesis genes. Therefore, millets growing in cold semi-arid regions exhibit altered starch content and composition in their grains, as compared to growth at normal conditions. Extensive research has been conducted on the proximate composition of millets from hot semi-arid regions, but research on millets from cold semi-arid regions is lacking. They need to be explored for their grain composition concerning climatic variations, as well as for their environmental adaptability, including parameters such as the endogenous level of hormones and its effect on grain starch. Studies on millets of cold semi-arid regions may unravel new germplasm with altered level of grain starch, amylose and protein. It will lead to a deeper understanding of cold temperature-mediated regulation of plant hormones for altered starch content and composition.

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