Changes in carbohydrate concentration in leaves, PODS and seeds of dry bean plants under drought stress

Article *in* Interciencia · March 2012

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Interciencia, vol. 37, núm. 3, marzo, 2012, pp. 168-175
Asociación Interciencia
Caracas, Venezuela

Interciencia
ISSN (Versión impresa): 0378-1844
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Asociación Interciencia
Venezuela

CHANGES IN CARBOHYDRATE CONCENTRATION IN LEAVES, PODS AND SEEDS OF DRY BEAN PLANTS UNDER DROUGHT STRESS

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SUMMARY

Changes in the concentration of sucrose, glucose, fructose and starch, in leaves, seeds and pods of Phaseolus vulgaris *L. cv 'Otomí' of determinate growth habit, were evaluated under both drought stress and non stress (irrigated) conditions. Plants were submitted to drought stress during the flowering, pod formation and seed filling stages. The stress increased the concentrations of glucose from 18 up to 196% and of fructose from 20 up to 333% in mature leaves, likely due to starch hydrolysis, while showing starch reductions from 17 up to 46% and sucrose reductions from 41 up to 70%. These changes in leaves promoted the sugar exports toward pods and seeds, since their sucrose concentration increased. In the reproductive organs, the concentration of glucose and fructose of stressed plants increased 136 and 138% in pods and 83 and 73% in seeds, respectively. Contrary to the changes registered in leaves, in reproductive tissues the sucrose increased up to 238% in pods and up to 148% in seeds, while the starch became reduced down to 37% in pods and to 21% in seeds. The increase of hexoses in pods and seeds might be explained by in situ starch hydrolysis, whereas the increase in sucrose is probably due to an enhanced synthesis of this sugar for a higher osmotic adjustment.*

Water is the most restrictive factor for yield in common bean (*Phaseolus vulgaris* L.) (Cuellar-Ortiz *et al*., 2008, Jie *et al*., 2010); however, plants have developed several mechanisms that allow them to survive under environmental stress conditions, such as morphological and physiological changes in vegetative tissues or in seeds (Caruso *et al*., 2002). The morphological mechanisms are: escape, by flowering to com-

KEYWORDS / Drought Stress / Fructose / Glucose / *Phaseolus vulgaris* / Starch / Sucrose /

Received: 02/17/2011. Modified: 02/17/2012. Accepted: 02/22/2012.

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plete life cycle before drought; avoidance, by reducing water loss from plants, due to stomatal control of transpiration, and maintaining water uptake through an extensive and/or deep root system (Farooq *et al*., 2009); phenotypic flexibility, generally by limiting the leaf number and size in response to drought stress (Schuppler *et al*., 1998); and molecular mechanisms, changes in gene expression (up-down regulation). The most important physiological mechanisms for drought tolerance include: osmotic adjustment, osmoprotection, antioxidation and a scavenging defense system (Farooq *et al*., 2009).

In different species, it has been shown that drought conditions affect the relationship between the carbon content in photosynthetic organs such as leaves (source), and the carbon content in heterotrophic organs such as seeds and roots (sink), thus indicating that the processes related to carbon partitioning are sensitive targets of this adverse environment (Cuellar-Ortiz *et al*., 2008). In this context, Reguera *et al.* (2011) have shown that the expression analysis and enzyme activities of several genes are associated with carbon and sugar metabolism. Under water deficit, sugars replace water molecules and form hydrogen bonds with other molecules that interact with polar groups, avoiding conformational changes and maintaining enzymatic activity (Yordanov *et al*., 2003).

However, the effect of drought stress on carbohydrates varies among species and plant tissue; for instance, a drought stress imposed on dry bean plants during the seed filling period did not alter the leaf concentration of fructose and glucose (Brevedan and Egli, 2003), whereas in leaves, flowers and pods of soybean (*Glycine max* L.(Merr.)) both fructose and glucose increased (Liu *et al*., 2004). The role of reducing sugars (glucose and fructose) in the adaptive mechanism is controversial, and even their accumulation can be detrimental from different perspectives (Kerepesi and Galiba, 2000).

The water deficit causes different responses in plants and tissues. For example, Rangel-Fajardo *et al.* (2011) reported that in cocoa (*Theobroma cacao* L.) seeds dried at 20-25°C until they contained 300g H_2O/kg fresh weight caused a reduction in sucrose accumulation; in contrast, the water stress induced an increase in the sucrose content in ovaries of maize (*Zea mayz* L.; Zinselmeier *et al.,* 1995) and rice (*Oryza sativa* L.; Wei *et al*. (2004)), in seeds of fava bean (*Vicia faba* L.; Lahuta *et al*. 2000), in leaves of cucumber (*Cucumis sativus* L.; Drozdova *et al*., 2004), and in rice stems (Wei *et al*., 2004). These increases in sucrose content have been attributed mainly to low activity of acid invertase (D-fructofuranosidase, EC3.2.1.2.5), which catalyzes sucrose hydrolysis to glucose and fructose (Zinselmeier *et al*., 1995; Stancato *et al*., 2001; Liu *et al*., 2004). Sucrose accumulation has been also correlated with activity of sucrose phosphate synthase (EC2.4.1.14), which plays a major role in sucrose biosynthesis (Baxter *et al*., 2003). In contrast, some authors have reported increases of sucrose and some hexoses due to increases in starch hydrolysis (Drozdova *et al*., 2004).

The effect of sucrose in the metabolic process is important because it is the main sugar transported in plants (Bewley, 2001) and can be hydrolyzed into fructose and glucose by invertase (Li *et al*., 2012). Basu *et al*. (2007) consider that sucrose and some hexoses participate in osmotic adjustment, and have proposed that these changes in carbon partitioning could contribute to osmoregulation. Sucrose can replace water in order to maintain membrane phospholipids in the liquid-crystalline phase and to prevent structural changes in soluble proteins (Kerepesi and Galiba, 2000).

Starch is the main stored carbohydrate reserve in higher plants, formed of two polymers of glucose, amylopectin and amylase. Starch can be found in fruits, seeds, rhizomes and tubers, because it can be synthesized in plastids of both photosynthetic and non photosynthetic cells as a result of photosynthesis during daytime; it can be stored in the chloroplast, and then degraded to triose phosphates for being exported to the cytosol, where it is used as a substrate for the synthesis of sucrose or as a source of energy. As the principal carbohydrate storage, starch plays important roles during the life cycle to support new growth and development of the plant (Grennam, 2006; Zeeman *et al*., 2010).

Some authors have reported reductions in the starch concentration in leaves of soybean (Liu *et al*., 2004), dry bean (Brevedan and Egli, 2003) and chickpea (*Cicer arietinum*; Basu *et al*., 2007). In sink organs, however, the starch concentration increases in pods and flowers of soybean (Liu *et al*., 2004) and in flowering and post-flowering plants of *Cassia angustifolia* Vahl (Agarwal and Pandey, 2002). According to the later authors, starch increases are due to a decrease of activity of α-amylase, which is the main starch degrading enzyme. Other authors have reported reductions in seeds of *V. faba* (Lahuta *et al*., 2000) that have been attributed to decreases in sucrose synthesis and acid-invertase activity and, consequently, in the synthesis of reducing sugars required for starch synthesis.

In the present study the concentration of soluble sugars and starch were investigated in leaves, seeds and pods of a common bean cultivar subjected to drought stress and irrigation conditions during different reproductive stages.

Materials and Methods

The study was conducted under greenhouse conditions at Montecillo, State of Mexico, using the dry bean cv. 'Otomí' of determinate growth habit. This variety is cultivated in semi-arid highland regions because of its short growing season and good adaptation to low-raining conditions with an annual average precipitation of 200-400mm. Seeds were planted into 6 liter plastic containers filled with a mixture of loam soil, river sand, peat moss and agrolite (2:2:1:1). Field capacity (FC) and permanent wilting point (PWP) of the substrate were determined through the pressure pot and the pressure membrane, respectively, and a moisture retention curve was generated (Figure 1).

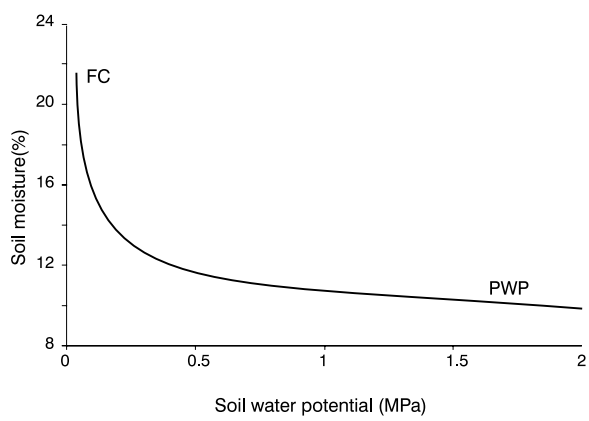

Figure 1. Soil moisture retention curve of the substrate as determined through the pressure pot and the pressure membrane, showing the field capacity (FC) and the permanent wilting point (PWP).

Drought stress treatments were applied at: 1) flowering stage (FS), R6 stage; 2) at pod formation (PFS), R7 stage; and 3) at seed filling (SFS), R8 stage; and were compared to a control under irrigation (I). The stress treatments consisted of interrupting irrigation until reaching PWP (11.5% of substrate moisture content) plus 10 days under PWP; at the end of the stress period irrigation was resumed at regular intervals. The control was kept near field capacity (22.5% of substrate moisture content). Leaf and pod water potentials (Ψ_l and Ψ_p) were determined at each stage, using a Scholander pump model A699 (Soil Moisture Equipment Corp. Santa Barbara, CA, USA).

All treatments were distributed under a randomized complete block design with three replicates, using a group of five pots with a single plant per pot as experimental unit. During plant development each pot was weighed daily and the amount of consumed water was estimated by the difference of weights at consecutive days; then, using the moisture retention curve, the required amount of water for maintaining the substrate at field capacity was supplied through irrigation, except during stress periods. Mean temperature and relative humidity in the greenhouse during the growth season ranged from 17 to 23°C and from 57 to 75%, respectively.

Tissue collection

In order to determine carbohydrate contents, five mature leaves from the upper four trifoliate leaves per replicate, were collected per replicate in all treatments, after 0, 6 and 10 days of withholding irrigation. Pods were collected at 4, 7, 10, 14, 18, 22 and 30 days after floral opening (DAF). The samples were frozen in liquid nitrogen and stored at -20°C. Pods 2-10 days old were collected in the PFS treatment, and pods 10-22 days old in the SFS experiment.

Extraction and quantification of soluble sugars

Soluble sugars were extracted from pods, seeds and leaves by four to six successive 10min incubations with hot ethanol (80%, v/v, 70°C). Previous tests had shown that no further sugars were released by additional ethanol incubations. The extracts were evaporated at 50°C, then re-suspended in distilled water and stored at -20°C until analysis. Glucose (Glc), fructuose (Fru) and sucrose (Suc) were assayed after the sequential addition of hexokinase, (EC 2.7.1.1), phosphoglucose-isomerase (EC 5.3.1.9) and invertase (EC 3.2.1.26). The assay was linked to the activity of glucose-6-phosphate dehydrogenase (G6PD; EC 1.1.1.49) in a two step assay measuring NADH formation at 340nm in a microplate reader (Multiskan Ascent, Thermo Labsystems; Scholes *et al*., 1994). In the first part of the assay, the ethanol extract was mixed with 100mM HEPES buffer (pH 7.5), 40mM NAD (Sigma), 100mM ATP pH 7 (Sigma) and 0.05U·ml^{-1} G6PDase (Roche). In the second part of the assay, glucose concentration was determined by the addition of 10µl of 0.05U·ml^{-1} hexokinase (Roche) and allowed to react for 20min prior to reading at 340nm. Fructose was determined after adding 10µl of 0.06U·ml^{-1} phosphoglucose isomerase (Roche) and 20min later reading was taken. Finally, the sucrose concentration was determined by adding 10µl of 0.8U·ml^{-1} invertase (Sigma) and the reading taken after 20min.

The remaining tissue, insoluble in ethanol, was used for starch determination. This insoluble tissue was homogenized in 1ml of distilled water and autoclaved for 30min. Starch content was determined in this mixture by incubating 100µl for 4.5h at 37°C with a buffered solution of hydrolytic enzymes (100µl of 500mM MES-KOH, pH 4.5; 50µl of 4mg·ml^{-1} amyloglucosidase (EC 3.2.1.3); and 50µl of 90mg·ml^{-1} α-amylase (EC 3.2.1.1) and assaying for Glc as described above (Scholes *et al*., 1994).

Starch grain identification by light microscopy

In the drought stress treatment imposed during flowering, floral-buds and pods of 2, 1, and 0 days prior to floral opening were collected to observe starch grain accumulation by light microscopy. To visualize the starch, the inclusion in paraffin technique was used. Briefly, tissue was fixed in ethanol 80% (v/v), followed by dehydration (dehydrator Mod. 4640-B) for 12h. Then, 5µm thick sections were obtained with a microtome and dyed with I_2-KI (0.20% (w/w) I_2 and 0.53% KI) and observed through a light microscope (Zeiss Model 34936).

The data were analyzed with the SAS (Statistical Analysis System) program version 6.12, through analysis of variance and multiple mean comparisons (Tukey, $P<0.05$).

Results and Discussion

Water status in leaves and pods

At the end of the drought stress treatments, after 10 days under water stress, the average values for Ψ_l were -1.1MPa in FS, -1.1MPa in PFS, -1.2MPa in SFS, and -0.64MPa under irrigation (I). The values for Ψ_p were -1.2 and -1.5MPa for PFS, SFS, and -0.7MPa under I. It is clear that drought caused reductions in Ψ_w in both tissues compared to the control, but in pods the values were lower than in leaves, probably due to a better osmotic adjustment, as proposed by Turner and Jones (1980).

Similar reductions in Ψ_l have been reported by other authors. Acosta and Kohashi (1989) reported a Ψ_h of -1.5MPa in leaves of dry beans subjected to drought stress for 15 days, from the beginning of the flowering phase. Ma *et al*. (2001) registered a Ψ_l of -1.4MPa in chick-pea pods (*Cicer arietinum* L.) after being subjected to drought stress during 10 days. According to Miyashita *et al*. (2005) the physiological recovery of dry bean plants subjected to drought stress of -0.6MPa is 100%, but under -1.2MPa the recovery reaches only 80, 60, and 40% for photosynthesis rate, transpiration rate, and stomatal conductance, respectively. In maize, Schussler and Westgate (1991) considered that Ψ_l values of -0.7 and -1.1MPa during flowering correspond to a moderate and severe drought stress, respectively. Therefore, the stress applied to dry bean plants in the present study might be considered between moderate and severe.

Glucose, fructose, sucrose, and starch concentrations

Mature leaves. In FS, glucose content in leaves increased 1.88 (78.5%) and 2.12 (196%) mg·g^{-1} of fresh weight (FW) at the 6th and 10th day of drought stress, whereas fructose increased 1.34 (190%) and 2.12 (333%) mg·g^{-1}·FW, respectively, compared to irrigated plants (Figures 2a and d). In contrast, sucrose and starch concentrations declined 1.21 (70%) and 19.1 (17.3%) mg·g^{-1}·FW (Figures 2g and j). A similar trend was observed for glucose and fructose in PFS, with increases of glucose of 2.85 (96%) and 2.27mg·g^{-1}·FW (128%), and fructose of 2.35 (96%) and 1.70mg·g^{-1}·FW (205%) on the 6th and 10th day of drought stress, respectively (Figures 2b and e); in this developmental stage, the concentrations of sucrose and starch declined 1.23 (56%)

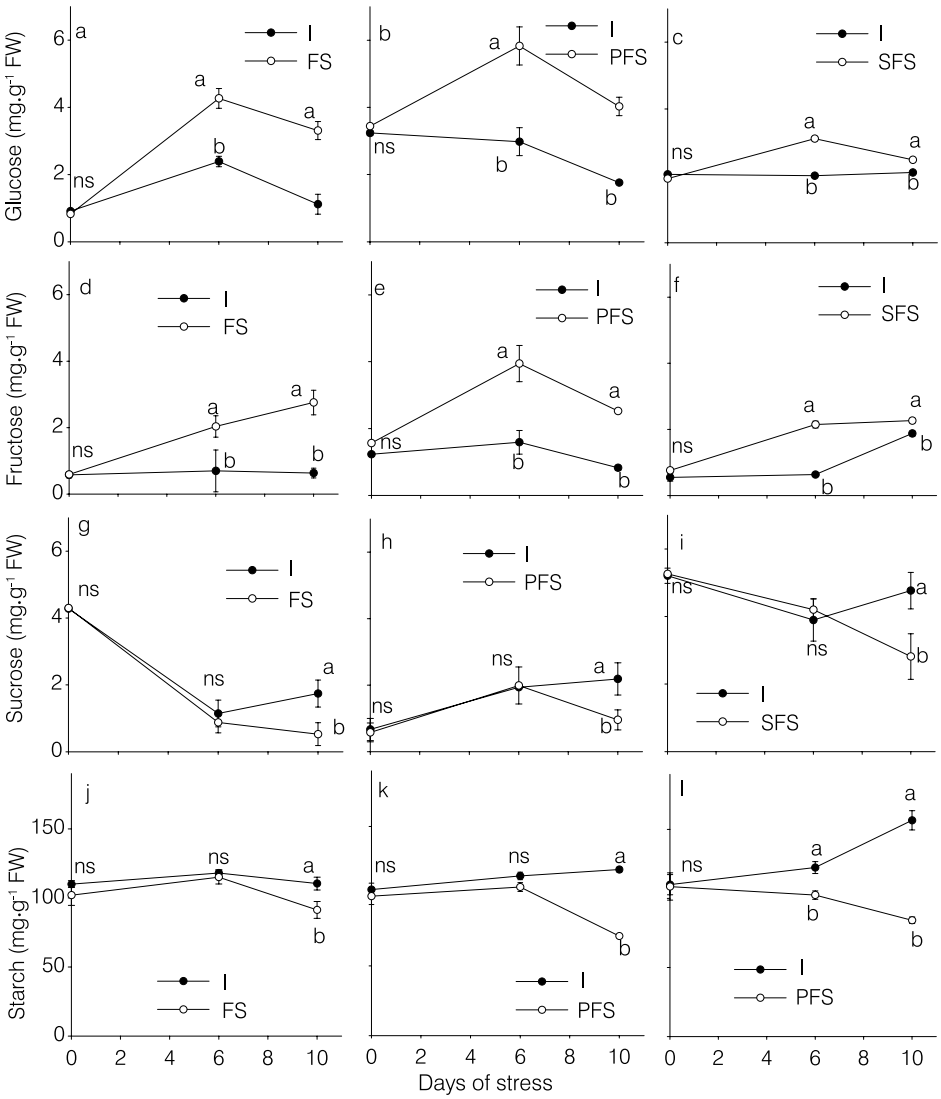

Figure 2. Concentrations in mg·g^{-1} FW of glucose (a, b and c), fructose (d, e and f), sucrose (g, h and i), and starch (j, k and l), in leaves of common bean plants subjected to drought stress at flowering (FS), pod formation (PFS), and seed filling (SFS) stages, compared to the irrigated control (I). Means (n= 3) with the same letter within a column are statistically equal (Tukey, 0.05; ns: non significant). Vertical lines are standard errors.

and 47.66 (40%) mg·g^{-1}·FW, respectively (Figures 2h and k). In SFS glucose concentration increased 1.11 (55%) and 0.38 (18%) mg·g^{-1}·FW and fructose 1.52 (228%) and 0.39mg·g^{-1}·FW (20%) after 6 and 10 days of drought stress, respectively (Figures 2c and f), while the concentrations of sucrose and starch declined 1.95 (41%) and 72.3 (46%) mg·g^{-1}·FW, respectively (Figures 2i and l). Cuellar-Ortiz *et al.* (2008) also reported a reduction of starch content in the leaves of a drought-resistant bean cultivar submitted to stress during the grain-filling stage. Patakas *et al.* (2002) showed that the starch concentration decreased almost threefold in leaves of drought-stressed grapevines (*Vitis vinifera* L.).

The increases in fructose and glucose registered in this study are in agreement with the findings of Liu *et al.* (2004) in leaves of soybean plants and Sato *et al.* (2004) in shoots of cabbage plug seedlings subjected to drought stress. Jie *et al.* (2010) also reported increases in glucose in leaves of apple (*Malus domestica*) seedlings subjected to drought stress. In the present study the increases in glucose on the 6th and 10th day were of 78 and 196% during FS; 96 and 128% in PFS; and 55 and 18% in SFS. The corresponding increments in fructose on the same days were of 190 and 333% in FS; 96 and 205% in PFS, and 228 and 20% in SFS. These increases in glucose and fructose were lower at the 6th than at the 10th day in all drought treatments, with the exception of SFS.

On the 10th day under drought stress the bean leaves maintained a higher concentration of these hexoses (glucose and fructose) than under irrigation, while the sucrose and starch concentrations decreased markedly. These results indicate that a moderate drought stress (1st to 6th day) promotes starch hydrolysis in leaves, perhaps due to stimulation of the α-amylase, which would explain the increases in glucose and fructose. Regardless of these changes, leaves were able to maintain higher concentrations of fructose and glucose under drought stress than under irrigation, assumedly to maintain a high turgidity during the drought stress, as it was demonstrated by Turner (1997), mainly at the flowering and pod filling stages (FS and PFS).

Taking into account these results, it could be inferred that the oldest leaves in the SFS treatment should have had a lower turgor than the mature ones evaluated in FS and PFS. The low response in the seed filling stage (SFS) could be attributed to the more advanced age of leaves, near to abscission, as proposed Brevedan and Egli (2003). According to Dungey and Davies (1982), older leaves are more susceptible to drought stress than young leaves.

The reduction of sucrose in leaves might indicate that sucrose synthesis had become reduced, so that drought stress not only limits the size of the source and sink tissues, but phloem loading, translocation of assimilates and dry matter partitioning are also impaired. However, the extent of these effects varies within plant species, stages, duration and severity of drought (Farooq *et al.*, 2009). The present results suggest that the transport of sugars via phloem to the demand organs was not blocked, as it could be confirmed by sugar increases in these tissues, which in turn might promote inhibition of the acid invertase that hydrolyses sucrose, as it has been shown in several species (Zinselmeier *et al.*, 1995; Stancato *et al.*, 2001; Liu *et al.*, 2004).

The present results are also in agreement with the findings of Keller and Ludlow (1993) in pigeonpea (*Cajanus cajan* (L.) Millsp), a tropical grain-legume in which drought caused moderate increase in glucose and fructose concentrations as well as a pronounced increase in the activities of enzymes hydrolyzing soluble starch (amylases) and sucrose (invertase and sucrose synthase), so that the sucrose content of leaves became diminished. Chen *et al.* (2007) also observed that drought increased glucose and fructose concentrations of carrot (*Daucus caro-*

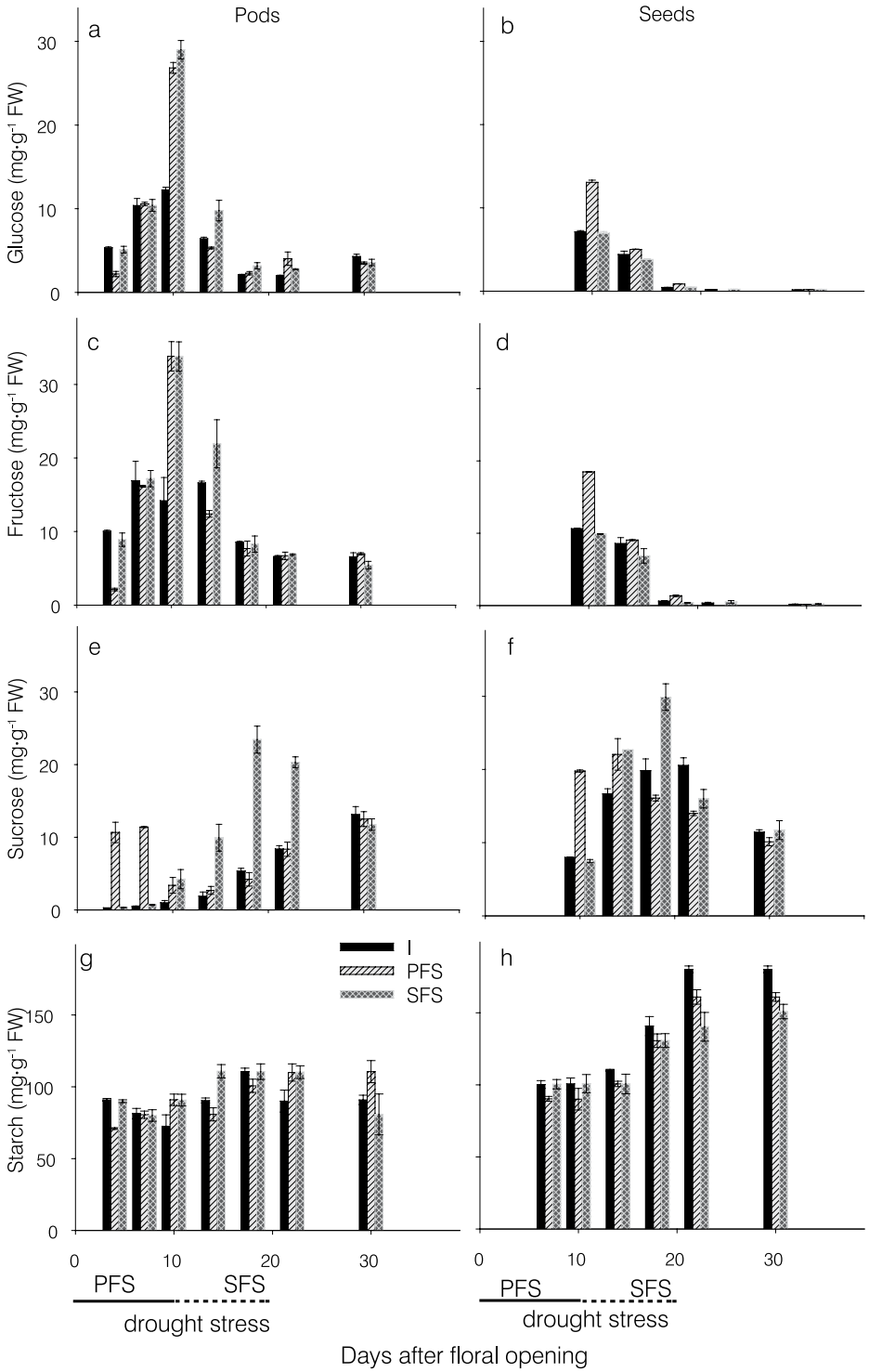

Figure 3. Concentration in mg·g^{-1}·FW of glucose (a and b), fructose (c and d), sucrose (e and f), and starch (g and h) in pods (a, c, e, and g) and seeds (b, d, f, and h), of common bean plants subjected to drought stress during the pod formation (PFS) and seed filling (SFS) stages, compared to a control under irrigation. Thin vertical bars are standard errors (n= 3).

ta L.) leaves while the sucrose content decreased.

In adult maize leaves, however, gene expression of acid invertase showed an opposite response to drought stress (Kim *et al*., 2000). On the other hand, Trouverie *et al.* (2003) demonstrated that ABA and not sugars appear to be the main promoter of *Ivr2* vacuolar invertase expression in drought-stressed adult maize leaves. Apparently, at the whole plant level, a down regulation of acid invertase in reproductive structures along with a simultaneous up regulation of the enzyme in source organs (e.g., adult leaves) is induced by drought stress. This might be of particular significance for plant adaptation to carbohydrate starving under drought conditions. Elevated concentrations of glucose and fructose due to greater acid invertase activity in leaves could lead to maintain leaf turgor pressure, thus enhancing the probability of survival during a large period of drought stress (Liu, 2004).

Pods and seeds. As in mature leaves, fructose and glucose concentrations increased in pods and seeds of plants submitted to drought during PFS and SFS treatments. At PFS the increases in fructose and glucose of pods were 14.7 (119%) and 19.64 (138%) mg·g^{-1}·FW (Figures 3a and c), and in seeds were 5.95 (83%) and 7.85 (73%) mg·g^{-1}·FW, respectively (Figures 3b and d). In SFS the concentration of these sugars was drastically increased, reaching 16.8 (136%) for glucose and 19.6 (138%) mg·g^{-1}·FW for fructose in pods (Figures 3a and c), whereas in seeds the concentrations of those sugars were not affected (Figures 3b and d). Since these hexoses are not transported by the phloem, it can be assumed that drought induced starch hydrolysis in vegetative and reproductive tissues, thus increasing the solute concentration, as reported here.

Some researchers have postulated that the increases in sucrose and some hexoses should be due to a higher degree of starch hydrolysis (Drozdova *et al*., 2004), to facilitate osmotic adjustment. Osmotic adjustment is a mechanism to maintain turgor in plant tissues under osmotic stress; it involves the accumulation of a wide range of osmotically active molecules or ions, including soluble sugars, alcohols, proline, glycinebetaine, organic acids, and calcium, potassium and chloride ions, etc. As a result of solute accumulation, the cell osmotic potential is lowered, and water is attracted into the cell for turgor maintenance (Farooq *et al*., 2009). Osmotic adjustments have been closely associated to high rates of photosynthesis and assimilate partitioning for grain filling (Basu *et al*., 2007). In pods of soybean plants subjected to drought stress, Liu *et al.* (2004) reported increases of fructose and glucose.

Contrary to the observed decrease of sucrose in leaves, in young pods from the PFS treatment, drought stress caused an increase in su-

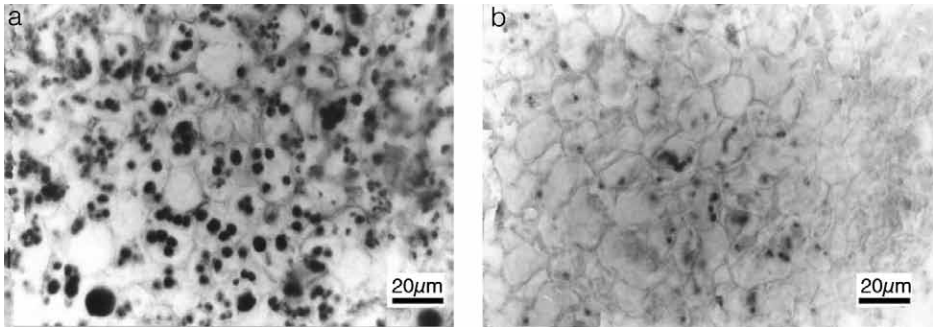

Figure 4. Starch grain accumulation in dry bean floral-buds two days prior to floral opening, under irrigated (a) and drought stressed (b) conditions. Starch grains in the basal section appear as brown dots.

crose content of 2.38 (238%) mg·g^{-1}·FW, while in SFS such increase reached a value of 11.9 (132%) mg·g^{-1}·FW at the end of the stress (Figure 3e). In the pod filling stage, the drought stress in seeds caused an increase in sucrose content of 11.8 (148%) mg·g^{-1}·FW; while in SFS the increase only reached 10.05 (50%) mg·g^{-1}·FW (Figure 3f). According to Basu *et al*. (2007), sucrose accumulation increases the gradient for water flux into the cell and maintains turgor by adjusting the leaf water potential and relative water content.

Even though it was not possible to quantify the starch content in FS due to insufficient reproductive structures, it was possible to detect visual differences in some tissues by histological analysis. In floral buds of two and one days before floral opening, and at the day of floral opening, it was evident that drought had caused reductions in starch grain accumulation at the basal and lateral portions of the pod (Figure 4). Starch reduction in reproductive structures due to water stress has been previously reported (Lahuta *et al*., 2000). In rice, the starch reduction has been attributed to the induction of α-amylase expression by drought stress, thus promoting starch hydrolysis (Wei *et al*., 2004; Zeeman *et al*., 2010). Basu *et al*. (2007) showed that a mild water stress helped in the conversion of starch into reducing sugars, followed by an increase in sucrose phosphate synthase that led to a transient accumulation of sucrose as an osmotic solute.

In young pods collected from the PFS treatment, starch increased by 27.2 (37%) mg·g^{-1}·FW (Figure 3g), whereas in their seeds it decreased by 16.8 (16%) mg·g^{-1}·FW (Figure 3h). No significant differences were found in starch concentration in pods from the SFS treatment (Figure 3g), but in seeds there was a significant reduction to 38.5 (21%) mg·g^{-1}·FW, compared to the irrigated control (Figure 3h). The reductions obtained in seeds in the PFS and SFS treatments are in agreement with the observation of histological preparations of floral buds (qualitative method; Figure 4b), Nevertheless, the starch increase observed by analytical techniques in young pods in PFS, diverge from the histological results where starch reduction appeared to occur; this is probably due to pod age differences; or the contrasting results indicate that the regulation of starch concentration in crop reproductive structures under drought is rather complex (Liu *et al*., 2004).

Under irrigated conditions, photosynthates are the primary suppliers of carbohydrates for ovary growth. Under a mild drought stress, however, the photosynthate flux from leaves decreases significantly, affecting ovary growth negatively (Liu, 2004). The sucrose increase reported above in sink organs should have been a result of sucrose synthesis in leaves from the glucose produced by starch hydrolysis, thus explaining the decline in sucrose and starch in leaves (Figures 2g to l).

Since pods were more affected by drought than their seeds, it may be inferred that pods act as a buffer cap for protecting the developing seed from severe metabolic changes, by remobilizing their carbon reserves to the seeds. In this regard, Yang *et al*. (2000) showed that water stress enhances remobilization of pre-stored carbon reserves to growing grains.

It is known that drought stress causes changes in the source-sink relationship, as well as in the partition between sucrose and starch in leaves, in order to favor the export of sucrose toward the sink organs, thus leading to a decline in sucrose and starch accumulation in leaves and to an increase of sucrose in various sinks (Basu *et al*., 2007). The drought effect in the partition between sucrose and starch was reported by Cuellar-Ortíz *et al*. (2008).

In the present study, the higher decline of starch in bean leaves in the stages of seed filling (46%) and pod filling (40%), as compared to the flowering stage (17%), may be attributed to a higher starch hydrolysis in order to favor sugar exports towards the reproductive organs that constitute the main sugar demand in these developmental stages. Starch is regarded as the main source of reserves in mature leaves (Hubert *et al*., 1984), by facilitating remobilization of leaf pre-stored photosynthates to various sinks (Basu *et al*., 2007). In pods collected at the seed filling stage only a slight reduction in starch concentration was observed, while in their seeds such a reduction reached 21% (Figures 3g and h), partly due to starch hydrolysis to obtain glucose for the synthesis of sucrose and some hexoses, as proposed by Drozdova *et al*. (2004).

The accumulation of sucrose recorded in bean pods and very young seeds during PFS, has also been reported in maize ovaries (Zinselmeier *et al*., 1995, 1999), in fava bean seeds (Lahuta *et al*., 2000), in soybean flowers and pods (Liu *et al*., 2004), and in common bean pods (Cuellar-Ortiz *et al*., 2008). Such accumulation in reproductive organs has been associated with a low activity of acid invertase in pods and with the respiratory demand in seeds (Stancato *et al*., 2001) and low activity of neutral invertase in common bean (Castrillo, 1992).

According to Basu *et al*. (2007), the increase in sucrose occurring in maize seeds was due to high starch hydrolysis and sucrose synthesis, which increased the solute concentration by osmotic adjustment as a mechanism of drought tolerance. It would also explain the scarce accumulation or even reduction in starch that took place in pods and seeds of dry bean in the present study. Drought stress stimulates the mobilization of soluble sugars to high demand organs, as Nerd and Neumann (2004) demonstrated in *Hylocereus undatus*, and as it was proposed by Zarco-Perelló *et al*. (2005) for maize. It could be inferred that in pods and seeds at the seed filling stage (more mature pods than in the previous treatments) the sink strength for sucrose had been reduced, thus explaining the reductions in sucrose and starch contents.

After irrigation was resumed in the drought stressed treatments, the concentration of all carbohydrates measured in this study in pods and seeds gradually increased until reaching, after 20 days, the control levels (Figure 3). These results indicate that once the bean tissues have recovered from the low water potential generated by drought stress, the metabolic processes in pods and seeds also recover. During recovery the sink organs show a faster increase in hexoses than in sucrose and starch. Recovery from the stress effects tended to be faster in the PFS treatment than in the SFS one, probably because young tissues have greater capacity of osmotic adjustment (Turner and Jones, 1980) than mature tissues for resuming the metabolic processes.

Conclusions

The drought stress applied at the stages of flowering, pod formation and seed filling increased the concentrations of glucose and fructose from 18 to 333% in mature leaves while starch decreased from 17 to 46%. This result is consistent with the hypothesis that starch hydrolysis increase leads to hexose accumulation. The sucrose accumulation was diminished by drought stress both in leaves and reproductive organs, due to the low photosynthesis and to the metabolic changes for keeping the sucrose transport in these adverse conditions. In pods of stressed plants, fructose and glucose concentrations increased, most likely due to starch hydrolysis, while the starch concentration became reduced up to 21% in seeds and up to 37% in pods, compared to unstressed plants.

ACKNOWLEDGMENTS

The authors thank Efraín Acosta Díaz, Campo Experimental de Calera, Zacatecas, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), for providing the seeds of the dry bean cultivar "Otomí".

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CAMBIOS EN LA CONCENTRACIÓN DE CARBOHIDRATOS EN HOJAS, VAINAS Y SEMILLAS DE PLANTAS DE FRIJOL NEGRO BAJO ESTRÉS HÍDRICO

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RESUMEN

*En el presente estudio se evaluaron los cambios, bajo irrigación y estrés hídrico, en la concentración de azúcares solubles: sacarosa, glucosa y fructosa, y de almidón, en hoja, semilla y vaina de frijol negro (*Phaseolus vulgaris *L) variedad 'Otomi', de crecimiento determinado. Las plantas fueron sometidas a estrés hídrico durante floración, formación de vainas y llenado de semillas. El estrés hídrico incrementó las concentraciones de glucosa desde 18 hasta 196% y de fructosa de 20 hasta 333% en hojas maduras, lo que se explica por hidrólisis del almidón, que mostró reducciones desde 17 hasta 46%, y por reducciones en sacarosa desde 41 hasta 70%, lo que a su vez favoreció las exportaciones a las vainas y semillas, en donde aumentó este azúcar de transporte. En los órganos reproductivos la concentración de glucosa y fructosa se incrementó en 136 y 138% en vainas y en 83 y 73% en semillas, respectivamente. A diferencia de lo que ocurrió en hojas, en los órganos reproductores la sacarosa se incrementó hasta 238% en vainas y en 148% en semillas; en almidón la sequía causó reducción de 37% en vainas y de 21% en semillas. Los incrementos en hexosas en vainas y semillas se atribuyen a hidrólisis del almidón, mientras que el aumento de sacarosa en vainas y semillas se atribuye probablemente a una síntesis mayor de este azúcar por un ajuste osmótico más alto.*

MUDANÇAS NA CONCENTRAÇÃO DE CARBOIDRATOS EM FOLHAS, VAGENS E SEMENTES DE PLANTAS DE FEIJÃO PRETO SOB ESTRESSE HÍDRICO

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RESUMO

*No presente estudo foram avaliadas as mudanças, sob irrigação e estresse hídrico, na concentração de açúcares solúveis: sacarose, glucose e frutose, e de amido, em folha, semente e vagem de feijão preto (*Phaseolus vulgaris *L) variedade 'Otomi', de crescimento determinado. As plantas foram submetidas a estresse hídrico durante floração, formação de vagem e enchimento de sementes. O estresse hídrico incrementou as concentrações de glucose desde 18% até 196% e de frutose de 20% até 333% em folhas maduras, o que se explica por hidrólise do amido, que mostrou reduções desde 17% até 46%, e por reduções em sacarose desde 41% até 70%, o que por outro lado favoreceram as exportações das vagens e sementes, o que aumentou este açúcar de transporte. Nos órgãos reprodutivos a concentração de glucose e frutose se incrementaram em 136% e 138% em vagens e em 83% e 73% em sementes, respectivamente. Diferentemente do que ocorreu em folhas, nos órgãos reprodutores, a sacarose se incrementou até 238% em vagens e em 148% em sementes; em amido a seca causou redução de 37% em vagens e de 21% em sementes. Os incrementos mn hexosas em vagens e sementes se atribuem a hidrólises do amido, enquanto que o aumento de sacarose em vagens e sementes se atribui provavelmente a uma síntese maior deste açúcar por um ajuste osmótico mais alto.*