EUROSOYA N° 4, 1986

Effects of cold stress on carbohydrate content in leaves with relation to cold tolerance of two soybean strains

M. RINDLISBACHER (†), E.R. KELLER, A. SOLDATI, P. SCHLEPPI

Department of Crop Science-Swiss Federal Institute of Technology - 8092 Zürich, (Switzerland).

SUMMARY

The nonstructural carbohydrate contents (starch and free sugars) of leaves and their specific weights were determined for soybean plants grown in controlled environment chambers and subjected to a cold stress. Two strains considered to be cold sensitive and cold tolerant respectively were studied. A temporary increase in the free sugar contents caused by chilling was shown at the beginning of flowering and during seed filling. At the beginning of flowering, a greater accumulation of foliar starch occurred during the first light period under low temperature. After several days, the main effect of the stress was an increase in the specific leaf weight. As a result of the slight effect of a 9 day cold stress on the grain yield and the lack of clear interactions between temperature and genotype for the studied parameters, it was not possible to propose indirect screening criteria for cold tolerance during flowering.
Additional key words : *Glycine max* (L.) Merr., chilling, starch, free sugars, specific leaf weight.

RESUME

Effets d'un stress de froid sur les glucides foliaires - Relations avec la tolérance au froid de deux lignées de soja.

Les teneurs en glucides non structuraux (amidon et sucre soluble) des feuilles et leur masse spécifique ont été mesurées sur des plantes de soja cultivées en chambre climatisée et soumises à un stress de froid. Deux lignées préalablement jugées respectivement sensible et tolérante au froid ont été étudiées. Une augmentation temporaire des teneurs en sucres due au froid a été démontrée en début de floraison et pendant le remplissage des grains. Au premier de ces stades, une plus forte accumulation d'amidon foliaire a eu lieu pendant la première période de lumière à basse température. Après plusieurs jours, le principal effet du stress a été une augmentation de la masse foliaire spécifique. Le faible effet d'un stress de 9 jours sur le rendement en grains, ainsi que le manque d'interactions nettes entre température et génotype quant aux paramètres étudiés, font qu'aucun critère indirect de sélection pour la tolérance au froid pendant la floraison ne peut être proposé sur la base de ce travail.

Introduction

Temperature is a main limiting factor for the extension of soybeans into cool temperate regions (SOLDATI and KELLER, 1985). Cold tolerance at different stages of growth is therefore an important aim in breeding soybeans for these areas.

Genetic variability already exists for this character (HOLMBERG, 1973; LITTLEJOHNS and TANNER, 1976; SCHMID and KELLER, 1980). However, cold tolerance is not a well defined varietal trait: it depends on the moment (stage of plant growth), intensity (temperature level) and duration of cold weather periods (SCHMID and KELLER, 1980). We have to distinguish especially between cold tolerance during germination, vegetative growth, flowering and pod filling. This means that cold tolerance is not directly expressed in the field but is in interaction with precocity as well as seasonal and daily patterns of temperature and other ecological factors. Cold tolerance is therefore difficult to estimate from field trials, and it would be very useful to have at our disposal simple, reproducible and sure methods for screening soybeans. Such methods should predict behavior in the field at one or more growth stages.

All physiological processes vary more of less with temperature. It is therefore important to determine mechanisms responsible for cold sensitivity among the processes which are highly sensitive to temperature and are important for yield formation. Simplified screening tests would then have to be developed. Another approach might be to evaluate various morphological or biochemical traits for their correlation with cold tolerance without fully understanding cold stress physiology. Using this latter approach, we searched for possible indicators of cold tolerance in the variations in contents of leaf carbohydrates during a cold stress at the beginning of flowering. The starch and sugar contents of the leaves are indeed known to be modified in cold stressed plants (LEVITT, 1980), particularly in soybeans (BRENNER, 1984).

Material and methods

Plant material

Two soybean strains (*Glycine max* (L) Merr.) differing in cold tolerance were used in our experiments. Strain S31 192/20267 (= ISZ-7 x Maple Arrow; number refers to the soybean breeding program of the Swiss Agricultural Research Station in Changins) was reported to be cold tolerant because it set pods when grown at 15/9 °C. day/night temperatures (from the first trifoliate stage on); strain S31 482/20303 (= K 312-202 from the Agriculture Canada Research Station in Ottawa) failed to set pods under the same temperature conditions and was reported to be cold sensitive (ROSSIER and UEHLINGER, pers. comm., 1983).

Plant culture and temperature treatments

In our first two experiments, plants from inoculated seeds were grown in clay pots (16 cm. diameter) containing field soil. Pots, were placed in five randomized blocks in a controlled environment chamber (Therma Weiss 10E/JUPK) set at 22/17 °C. day/night temperatures, 70 to 80 % RH, 16 h. daylength and a light intensity of about 17 klux (80 % fluorescent and 20 % incandescent). Plants were watered daily. At the beginning of flowering (stage R1, FEHR and CAVINESS, 1977), plants were transferred into two Frigorex chambers (type E15), the first having the same environmental conditions (22/17 °C. day/night temperatures) as the Therma chamber (for control plants) and the second with day/night temperatures of 12/7 °C. (cold-stressed plants). In the first experiment, the stress began at the end of the dark period or at the end of the light period and lasted 24 h. In the second experiment, a cold stress of 9 d. was applied beginning with a night period. In a third experiment, we studied (in eight replicates) the effects of a 24 h. cold stress (11/6 °C. vs. 22/17 °C.) on strain 20303 during seed filling (stage R 5.5).

Sampling procedures

Two leaves, just having reached full expansion, were used for chemical analyses. Before analysis, leaf area was measured and the leaves dried at 105 °C. for 1 h. and then at 60 °C.. In the first experiment, samples were taken a) just before the stress began, b) at the end of the subsequent light or dark period and c) after 24 h. of cold exposure. In the second experiment, the rest of the shoot was also harvested and dried at 65 °C.. Six harvests were made at the beginning and at the end of the light periods a) before the stress, b) after 5 d. for the control and c) after 9 d. for the low temperature treatment (the sum of the temperatures above 0 °C. between the beginning of the stress and the harvests was about the same i.e. 2350-2600 deg. h). The number of grains per plant was determined at maturity in a final harvest. In the third experiment, leaves were sampled every 4 h. from the onset of the treatments.

Carbohydrate analysis

The soluble sugar and starch contents were analyzed according to the procedures described by BRENNER (1984).

Statistics

Statistical interpretation of the data consisted of classical analysis of variance with, if required, comparison of treatment means by the DUNCAN test. The results obtained before the stress were not included in the overall analysis.

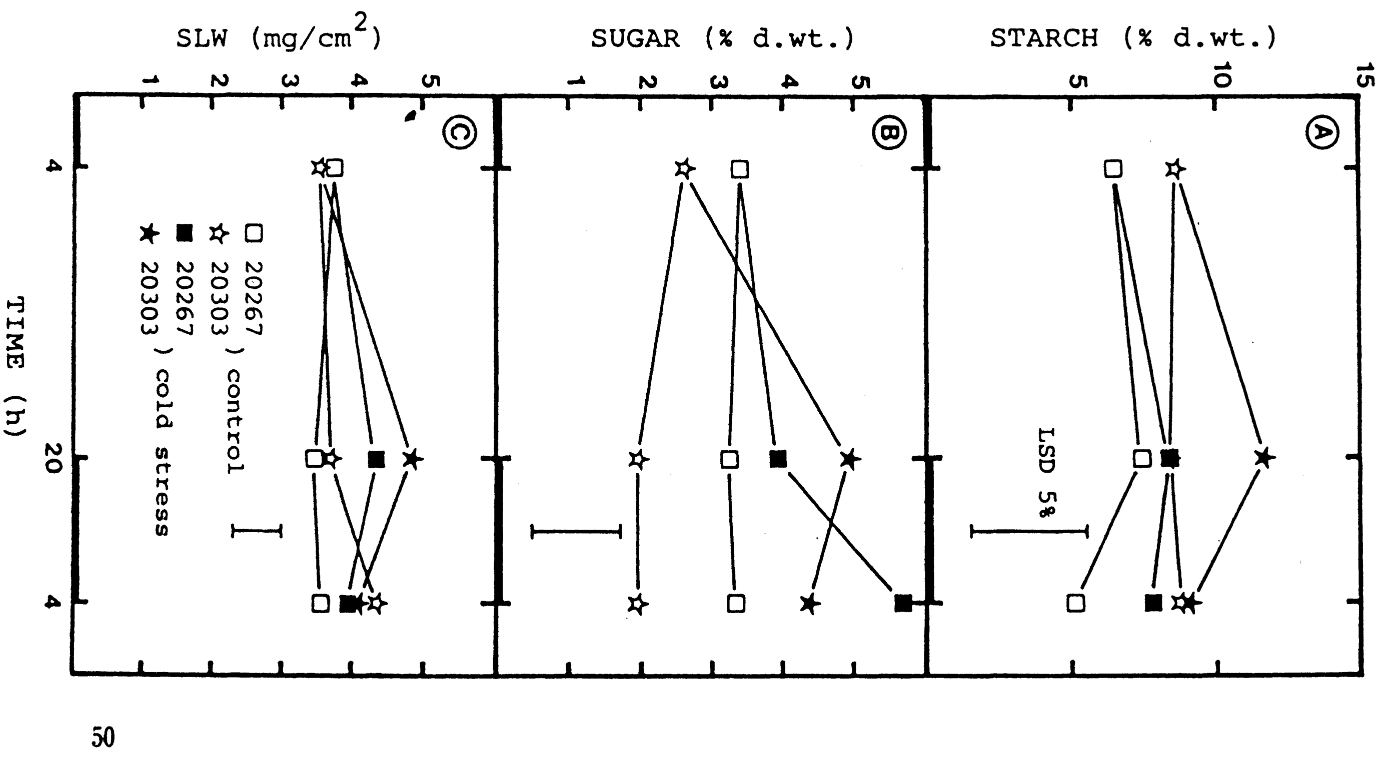
Results

24-h stress at the beginning of flowering

When not subjected to a cold stress, the two soybean strains had a TNC content of about 10 % in the leaves at the R1 stage partitioned as follows : more starch for strain 20303 (considered to be cold sensitive) and more free sugars for 20267 (fig. 1 and 2). The samples showed great variations in TNC, starch and specific leaf weight (SLW) values as well as between the two days on which the control was analyzed.

Figure 1 - Leaf starch Ⓐ and free sugar Ⓑ contents, and specific leaf weight (SLW,Ⓒ) of two soybean strains (20267 and 20303) at the beginning of flowering, for the 24 h. following the onset of a cold stress (12/7 °C vs. 22/17 °C) at the beginning of the light period. Means of 4 or 5 replicates ± least significant difference (LSD) at the 5 % level.

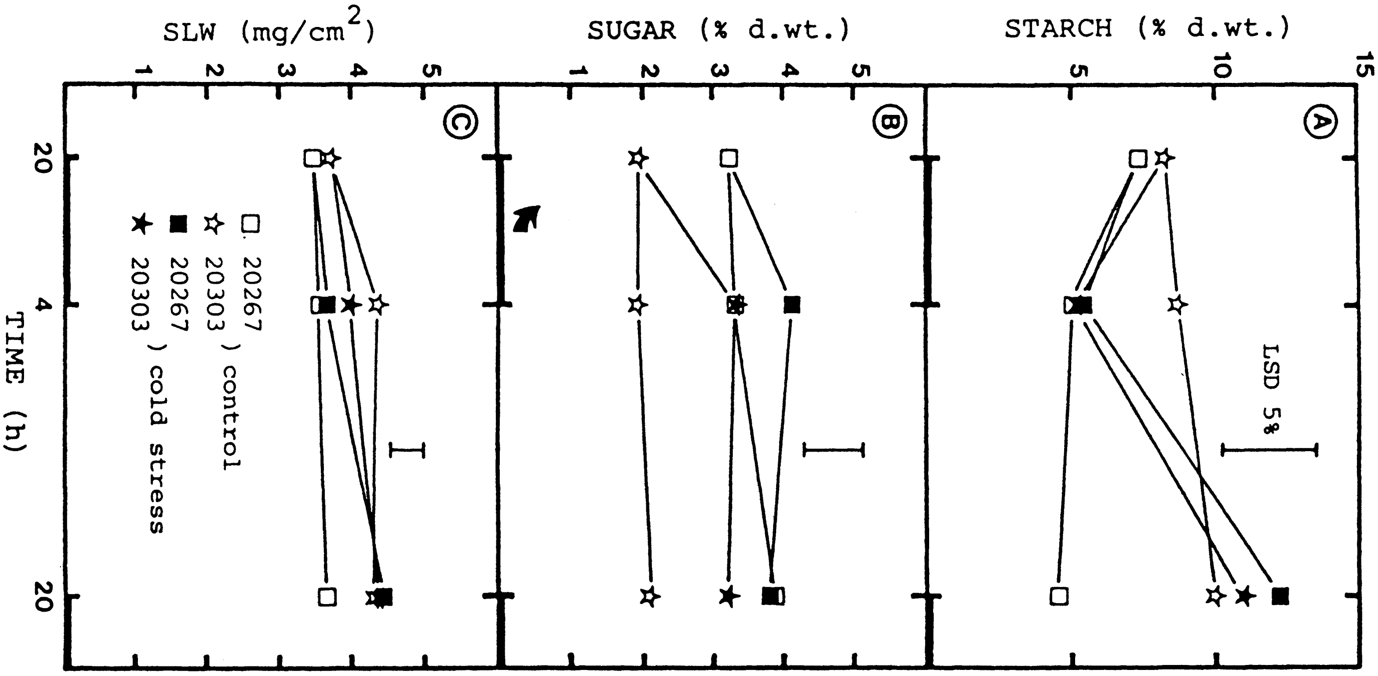
- Teneurs des feuilles en amidon Ⓐ et sucre soluble Ⓑ , et masse foliaire spécifique Ⓒ de deux lignées de soja (20267 et 20303) en début de floraison, pendant les 24 h d'un stress de froid (12/7 °C contre 22/7 °C) commençant au début de la période éclairée. Moyennes de 4 ou 5 répétitions ± plus petite différence significative (LSD) au niveau de 5 %.



A detailed analysis of these results is therefore not possible, but an overall increase in TNC, starch, and SLW could be observed during the light period following both stress treatments. Logically, the same is true when the leaf carbohydrates are expressed per unit area. Strain 20303 showed a rapid and important accumulation of free sugars during the cold treatment whereas strain 20267 showed a lesser increase which was not significant in all cases. This tendency of 20303 to accumulate more sugars than 20267 at low temperature is reflectd by an interaction between strains and treatments (statistically significant when both stress trials are computed together).

Figure 2 - Leaf starch Ⓐ and free sugar Ⓑ contents, and specific leaf weight (SLW,Ⓒ) of two soybean strains (20267 and 20303). at the beginning of flowering, for the 24 h following the onset of a cold stress (12/7 °C vs. 22/17 °C) at the beginning of the night. Means of 4 or 5 replicates ± least significant difference (LSD) at the 5 % level.

- Teneurs des feuilles en amidon Ⓐ et sucre soluble Ⓑ, et masse foliaire spécifique Ⓒ de deux lignées de soja (20267 et 20303) en début de floraison, pendant les 24 h d'un stress de froid (12/7 °C contre 22/17 °C) commençant au début de la nuit. Moyennes de 4 ou 5 répétitions.± plus petite différence significative (LSD) au niveau de 5 %.



9-d. stress at the beginning of flowering

The growth of the cold stressed plants was slightly more rapid when dry matter was expressed as a function of temperature sums (fig. 3), in spite of the low threshold chosen (0 °C.). Just before the beginning of the stress, the measured parameters were similar to those of the control in the first experiment, except for the leaf starch content which was higher for both strains in the second experiment (tab. 1).

After 5 d., the control plants showed increases in leaf TNC content in the morning, principally because of the higher starch level of 20267 and more free sugars for 20303. As compared with the controls which had similar sums of temperature, the 9 d. stressed plants had the same free sugar content but tendencially more starch in the morning. The main effect of this longer cold stress was a more rapid increase in SLW (with its effect on the TNC per unit area). Starch, sugar and SLW measurements gave no indication of an interaction between treatments and strains in this experiment.

Number of grains per plant at maturity was lessened by the cold stress: -15 % and -11 % for strains 20267 and 20303 respectively (fig. 4). Strain 20303 showed a greater reduction in number of grains on the main stem but this was compensated for by a much higher yield of the branches.

Figure 3 - Shoot dry weight of plants of two soybean strains at the beginning of flowering, 5 days later at the control temperature (22/17 °C), or after 9 days of cold stress (12/7 °C). Means of 5 plants harvested in the morning and five harvested in the evening, ± least significant difference (LSD) at the 5 % level.

- Masse sèche aérienne de plantes de deux lignées de soja en début de floraison, 5 jours plus tard à la température témoin (22/17 °C), ou après 9 jours de stress de froid (12/7 °C). Moyennes de 5 plantes récoltées le matin et 5 le soir, ± plus petite différence significative (LSD) au niveau de 5 %.

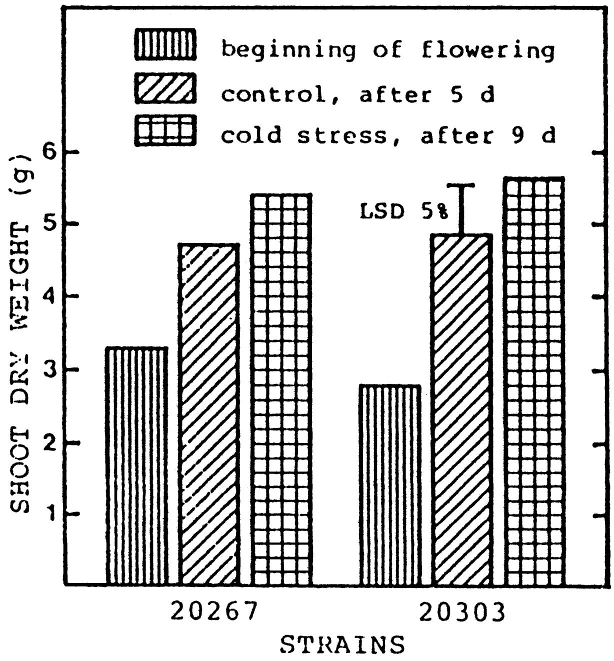


Table 1 - Leaf starch and free sugar contents, and specific leaf weight of two soybean strains after a cold stress of 9 d. Control plants harvested before the stress and after 5 d.

- Teneurs des feuilles en amidon et sucre soluble, et masse foliaire spécifique de deux lignées de soja après un stress de froid de 9 j. Plantes témoins récoltées avant le stress et après 5 j.

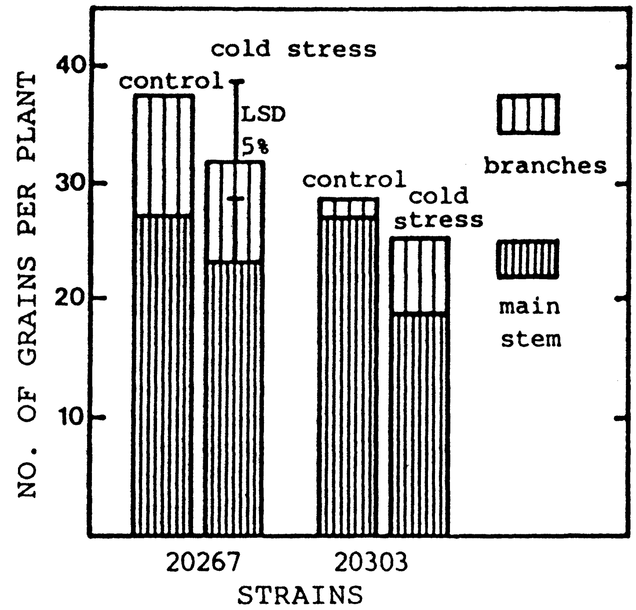
1) n.s. : not significant at the 5 % level
2) means followed by the same letter are not statistically different at 5 %

1) n.s. : non significatif au niveau de 5 %
2) les moyennes suivies de la même lettre ne sont pas statistiquement différentes au niveau de 5 %.

Strain	Treatment	Time	Sum of temperatures (degC·h)	Starch (%d.wt)	Sugar (%d.wt)	SLW (mg/cm^2)
20267	before	4 h	-352	8.4	3.75	3.54
	stress	20 h	0 (p.def.)	11.5	2.94	4.60
	control,	4 h	2576	13.8 n.s. 1)	3.92 n.s	5.32 a 2)
	after 5 d	20 h	2440	13.9 n.s.	3.43 n.s.	6.45 a
	cold stress,	4h	2368	15.6 n.s.	4.04 n.s.	7.83 b
	after 9 d	20 h	2560	14.7 n.s.	3.55 n.s.	8.35 b
20303	before	4 h	-352	11.3	2.59	4.47
	stress	20 h	0 (p.def.)	12.2	2.40	4.90
	control,	4 h	2576	11.6 n.s.	4.54 n.s.	6.39 a
	after 5 d	20 h	2440	12.7 n.s.	2.66 n.s.	5.69 a
	cold stress,	4 h	2368	14.8 n.s.	3.88 n.s.	8.37 b
	after 9 d	20 h	2560	13.1 n.s.	3.20 n.s.	8.71 b

Figure 4 - Number of grains harvested at maturity on the main stem and on the branches of two soybean strains subjected to a cold stress (12/7 °C vs. 22/17 °C) of 9 days at the beginning of flowering. Means of 5 replicates ± least significant difference (LSD) at the 5 % level.

- Nombre de grains récoltés à maturité sur les pousses principales et secondaires de deux lignées de soja soumises à un stress de froid (12/7 °C contre 22/17 °C) de 9 jours en début de floraison. Moyennes de 5 répétitions ± plus petite différence significative (LSD) au niveau de 5 %.

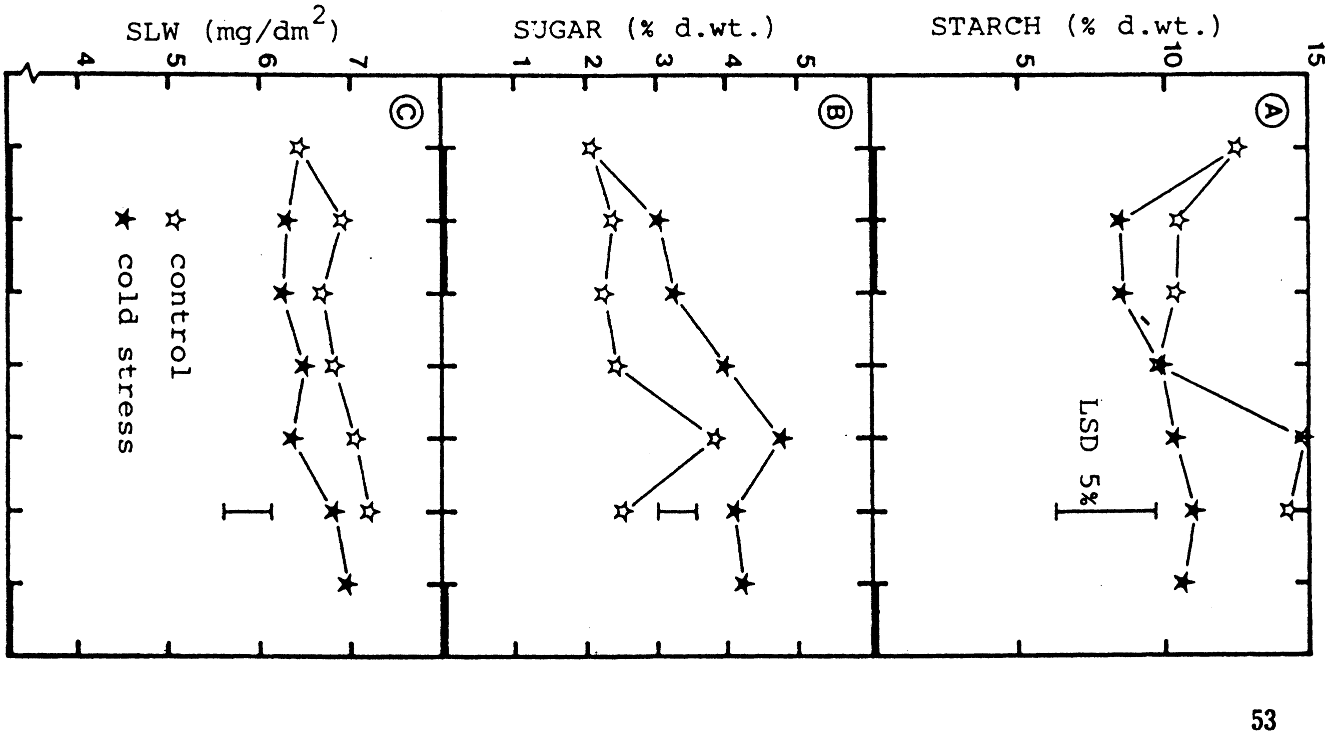


24-h. stress at seed filling

As at the beginning of flowering, the cold stress caused an increase in the free sugar contents of the leaves in strain 20303. After 4 h\, leaf dry matter was already higher by 0,5 % and statistically significant. Inversely, the starch contents were diminished by the stress (overall significant but not for each time of monitoring). During the middle of the light period (between 8 and 12 h.), a marked increase in the TNC contents of the leaves was observed for the control but not for the stress treatment. The SLW decreased after only 4 h of cold stress during the night.

Figure 5 - Leaf starch Ⓐ and free sugar Ⓑ contents, and specific leaf weight (SLW,Ⓒ) of soybean plants (strain 20303) during seed filling, for the 24 h following the onset of a cold stress (11/6 °C vs. 22/17 °C) at the beginning of the night. Means of 4 or 5 replicates ± least significant difference (LSD) at the 5 % level.

- Teneurs des feuilles en amidon Ⓐ et sucre soluble Ⓑ, et masse foliaire spécifique Ⓒ de plantes de soja (lignée 20303) en phase de remplissage des grains, pendant les 24 h d'un stress de froid (11/6 °C contre 22/17 °C) commençant au début de la nuit. Moyennes de 4 ou 5 répétitions ± plus petite différence significative (LSD) au niveau de 5%



Discussion

Our results show an altered carbohydrate metabolism in source leaves of soybean plants when exposed to a cold stress at the beginning of flowering. The observed short term increase in the free sugar contents is in agreement with a previous work on younger (stage V3) soybeans (BREENER, 1984). This seems to be explained by a greater starch degradation following a cold stress during the night. The biochemical equilibrium between starch and sugars is indeed well known to be modified, having more sugars at low temperature (see LEVITT, 1980). During the first light period, the faster accumulation of TNC in the leaves under stress indicates rather that the utilization of assimilates (translocation, respiration and growth) is affected to a greater extent than production (photosynthesis). This would mean that chilling temperatures have an effect either on translocatory paths or on carbon sinks. On the other hand, such an interpretation requires more support since carbohydrate partitioning in the leaves and export are not simply the results of independent processes of production and utilization, but are affected by a complex regulation system (GEIGER, 1979 ; HUBER and ISRAEL, 1982) capable of rapid adaptive changes (CHATTERTON and SILVIUS, 1980). The theory proposed by CHATTERTON and SILVIUS (1979) and by HEWITT et al. (1985) that the rate of diurnal starch accumulation by a plant is adapted to its night energy requirements is not confirmed by our results, where a colder night (then reduced metabolism and less energy demand) led to a faster starch accumulation during the next light period, in spite of a reduced day temperature. The tendency of our plants to have higher levels of starch in the leaves at the beginning of the light period following a cold stress of 9 d. than the approximately 4 days younger control plants (at approximately the same level of development) would better correspond to the above mentioned theory. However, the observed rates of diurnal starch accumulation are too low (or even negative) to allow a sure interpretation. These slight changes in the daily starch contents are in agreement with the conclusions of GIAQUINTA et al. (1985) who state that between anthesis and mid seed filling slowly turning over starch accumulates in the source leaves in addition to the daily turning over pool. It is noteworthy that the apparent tendency of 20303 to accumulate sugar instead of starch in the controls is not a statistically proven anomaly. The partitioning of TNC between these two carbohydrates shows important variations in the experiment described here. The higher SLW values under low temperatures are consistent with previous observations (unpublished) and reports (BRENNER et al., 1984; SATO, 1976). Our results show reduced expanding and/or exporting rates of the leaves during stress, suggesting also that the assimilate utilization is less affected by the cold stress than is photosynthesis.

At seed filling, the rapid increase in sugar contents at low temperature is explained by the parallel starch degradation. TNC accumulation during the day was lower than for the controls. If we assume that the translocation was decreased by the chilling at flowering, then this effect would disappear at the less cold sensitive stage of seed filling, eventually as a consequence of the greater sink mass and activity of the reproductive organs. Further investigations are required to test this hypothesis.

The initial goal of the present work was to find differences in the behavior of our strains under stress in order to use them in developing screening methods for cold tolerance. The only significant interaction between temperature treatments and plant genotypes was the greater increase in free sugar contents in our strain which had been judged previously to be cold sensitive. This significant result could only be otbained by combining two trials with 5 replications each. It is clear that the variability of this characteristic is much too high (relative to the observed differences) to be utilized as a screening criterion.

The ability of our strain 20303 to produce a yield following the stress treatment appears to be in contradiction to the previous observations of pod set failure at 15/9 °C., (ROSSIER and UEHLINGER, pers. comm., 1983). The duration of the stress is the only factor that can explain this discrepancy and we conclude that our low temperature exposure began too late and was too short in order to completely prevent the sensitive soybeans from setting pods. Moreover, the rather quick return to favorable growth conditions following the stress allowed the branches to compensate for the stress effect on the main stem. This confirms the importance of the time of the stress and its duration as already stated by SCHMID and KELLER (1980).

REFERENCES

BRENNER H., 1984. Einfluss kühler Temperaturen auf die oberirdischen Pflanzenteile und des Wurzel-systems bei der Sojabohne *(Glycine max.* (L.) Merr.) - Ein Beitrag über Kältetoleranz. Diss. ETHZ Nr. 7539.

BRENNER H., KELLER E.R., SOLDATI A., 1984. Effects of cool temperatures on the development of shoots, roots and reproductive organs of soybean plants. *Eurosoya* **2**, 24-28.

CHATTERTON N.J., SILVIUS J.E., 1979. Photosynthate partitioning into starch in soybean leaves. I. Effect of photoperiod versus photosynthetic period duration. *Plant Physiol.* **64**. 749-753.

CHATTERTON N.J., SILVIUS J.E., 1980. Acclimatation of photosynthate partitioning and photosynthetic rates to changes in length of the daily photosynthetic period. *Ann. Bot.* **46**, 739-745.

FEHR W.R., CAVINESS C.E., 1977. Stages of soybean development. Iowa State Univ. Agr. Home Econ. Exp. Stn. Spec. Rep. 80.

GEIGER D.R., 1979. Control of partitioning and export of carbon in leaves of higher plants. *Bot. Gaz.* **140**, 241-248.

GIAQUINTA R.T., QUEBEDEAUX B., SADLER N.L., FRANCESCHI V.R., 1985. Assimilate partitioning in soybean leaves during seed filling. pp. 729-738. In R. SHIBLES (ed.) *World Soybean Research Conference III: Proceedings.* Westview Press, Boulder and London.

HEVITT J.D., CASEY L.L., ZOBEL R.W., 1985. Effect of day length and night temperature on starch accumulation and degradation in soybean. *Ann. Bot.* **56**, 513-522.

HOLMBERG S.A., 1973. Soybean for cool temperate climates. *Agric. Hort. Gen.* **31**, 1-20.

HUBER S.C., ISRAEL D.W., 1982. Biochemical basis for partitioning of photosynthetically fixed carbon between starch and sucrose in soybean *(Glycine max.* Merr.) leaves. *Plant Physiol.* **69**, 691-696.

LEVITT J., 1980. Responses of plants to environmental stresses, vol. 1, Chilling, freezing, and high temperature stresses. Academic Press, New-York, 2nd ed.

LITTLEJOHNS D.A., TANNER J.W., 1976. Preliminary studies on the cold tolerance of soybean seedlings. *Can. J. Plant Sci.* **60**, 821-829.

SATO K., 1976. The growth responses of soybean plant to photoperiod and temperature. I. Responses in vegetative growth. *Proc. Crop Sci. Soc. Japan* **45**, 443-449.

SCHMID J., KELLER E.R., 1980. The behaviour of three cold tolerant and a standard soybean variety in relation to the level and duration of a cold stress. *Can. J. Plant Sci.* **60**, 821-829.

SOLDATI A., KELLER E.R., 1985. Soybeans adapted to cooler regions, pp. 460-467. In R. SHIBLES (ed.) *World Soybean Research Conference III: Proceedings.* Westview Press, Boulder and London.