

## Improving wheat grain filling under stress by stem reserve mobilisation

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### Summary

Stem reserves from pre-anthesis plant assimilation are being increasingly recognised as an important source of carbon for grain filling when current photosynthesis is inhibited by drought, heat or disease stress during this stage. Genotypic and environmental factors affecting reserve accumulation and utilisation for grain filling are reviewed. The genetic improvement of stem reserve storage and utilisation as a potent mechanism for grain filling under stress is discussed, and practical guidelines for selection work are provided.

### Introduction

In most wheat growing regions, and especially in the Mediterranean climate, grain filling is subjected to several physical and biotic stresses. Grain filling often occurs when temperatures are increasing and moisture supply is decreasing. Foliar diseases of wheat also tend to spread and intensify towards and after flowering. Leaf rust, stripe rust and septoria leaf blotch can result in total leaf destruction at grain filling. The common end-result of these stresses is kernel shrivelling, reduced test weight and loss in yield.

Current assimilation, as a source of carbon for grain filling, depends on the viable light intercepting green surfaces of the plant after anthesis. This source normally diminishes due to natural senescence and the effect of various stresses. At the same time the demand of the growing kernel increases, in addition to the demand due to maintenance and respiration of the live plant biomass.

Hence, an important source of carbon for grain filling is stem reserve. Even under mild conditions, current assimilates may be limited for normal grain filling. In a three year study in Connecticut (Gent, 1994), it was estimated that canopy respiration and grain dry matter accumulation sites were approximately equal sinks for photosynthates and, together, were greater than canopy photosynthesis late in

grain filling. Thus stem reserves are essential for completed grain filling.

While root storage is important in some legumes and other species, there is no evidence that roots or leaves are as important as stems for reserve storage in small grained species. Most studies of stem reserves in small grained species also include the leaf sheaths, as these contain reserves. The stems of small grained species store carbohydrates in the form of glucose, fructose, sucrose and starch, but the main reserve is fructan (e.g., Lopatecki et al., 1962; Dubois et al., 1990; Wardlaw & Willenbrink, 1994). Storage is commonly analysed as total non-structural carbohydrates (TNC) or water-soluble carbohydrates (WSC).

The first step, and probably the most important in fructan synthesis, is catalysed by the enzyme Sucrose:sucrose fructosyltransferase (SST). SST activity appears to be related to substrate (sucrose) concentration, which itself may be affected by sucrose synthase activity in the stem (Wardlaw & Willenbrink, 1994). Fructan accumulation is greater when sucrose is high in the penultimate internode of wheat (Dubois et al., 1990). Wardlaw & Willenbrink (1994) found that during the accumulation of WSC in wheat stems, the mass of fructan with a degree of polymerisation (DP) greater than five continued to increase, whilst the mass of fructans of DP 3-5 reached a maximum and then remained constant.

Fructan accumulated in the internodes during extension, although most of the fructan in an internode accumulated after it was fully extended. When WSC were mobilised from the stem, the mass of glucose, sucrose and fructan decreased but the mass of fructose first increased then decreased, indicating that fructan was hydrolysed at a faster rate than its product (Bonnett & Incoll, 1992b).

Starch is found in small amounts in wheat stems but is not mobilised, as evidenced from shading experiments (Kiniry, 1993).

### Reserve accumulation

Reserve accumulation and storage capacity in the stem strongly depend on the growing conditions before anthesis. Total stem TNC at anthesis was shown to vary from 50–350 g kg<sup>-1</sup> dry mass in different experiments (Kiniry, 1993).

Under optimal growing conditions with regard to temperature, water regime (Davidson & Chevalier, 1992) and mineral nutrition (Papakosta & Gagianas, 1991), carbon assimilation rates are high and a proportion of the assimilates is allocated to storage. When carbon assimilation during stem elongation is reduced by stress, storage in stems is reduced. For example, in water-stressed and irrigated wheat, remobilise WSC were measured at 641 mg and 1047 mg, respectively, because the former had less storage than the latter (Davidson & Chevalier, 1992). Under dryland field conditions, only half the amount of WSC were available for remobilisation during grain filling, compared with irrigated conditions. When the ambient CO<sub>2</sub> concentration was raised to increase assimilation, more carbon was stored in the stems (Winzeler et al., 1989).

Potential stem storage as a sink is determined by stem length and stem weight density. Stem weight density is equal to stem dry weight per unit stem length. Storage and remobilisation may vary along the stem. In winter barley, the basal internodes were found to contribute the most to grain filling (Bonnett & Incoll, 1992a). However, other studies with barley showed that the peduncle and penultimate internode (and leaf sheath) contained the most storage (Daniels & Alcock, 1982). Similarly in

wheat, the peduncle and the penultimate internode contained the most storage (Wardlaw & Willenbrink, 1994), with variations in storage and remobilisation under different experimental conditions being larger in the penultimate than in the fourth stem internode (Bonnett & Incoll, 1992a). Various aspects of stem anatomy with respect to storage were not thoroughly investigated except for the finding that there seems to be no consistent difference in total stem reserve accumulation between solid and hollow-stemmed wheats (Lopatecki et al., 1962).

Stem length is important in affecting stem reserve storage. The *Rht1* and *Rht2* dwarfing genes of wheat were found to reduce reserve storage by 35% and 39%, respectively, as a consequence of a 21% reduction in stem length (Borrell et al., 1993). However, under favourable conditions, the advantage of the tall genotype in reserve storage was not expressed as greater mobilisation to the ear. Under favourable grain filling conditions, only about 20% of grain yield was contributed by stem reserves in all genotypes. In barley, the contribution of stem reserves to grain yield was greater in a tall than in a short cultivar, but absolute yield was the same in both, indicating that the taller cultivar lacked in current assimilation compared to the shorter one (Daniels & Alcock, 1982).

### Reserve utilisation

Stem reserve mobilisation, or the percentage of stem reserves in total grain mass, is affected by sink size, by the environment and by cultivar. The demand by the grain yield sink is a primary factor in determining stem reserve mobilisation. When sink size was reduced by degrading, more reserves were stored in the stem, compared with intact ears (Kuhbauch & Thome, 1989). The interaction between ear size and the demand for stem storage appears to depend on the environment, before or during grain filling (Bonnett & Incoll, 1992a).

Environmental conditions that decrease current assimilation during grain filling cause a greater demand for stem reserves for grain filling. Shading of barley plants after anthesis promoted the use of stem reserves for grain filling (Bonnett & Incoll,

1992a). When wheat plants were shaded during grain filling, up to 0.93 g of grain was produced per gram of assimilates exported from the stem (Kiniry, 1993).

Stem reserve mobilisation is affected by water deficit during grain filling. Even the rate of development of water deficit may affect mobilisation. Palta et al. (1994) found that total grain carbon with a rapid water deficit rate was reduced by 24% relative to a slow rate. Post-anthesis assimilation was reduced by 57%, while remobilisation of reserves was increased by 36%. Interestingly, water deficit during grain filling also induced carbon mobilisation from tillers to the main stem ear.

It is therefore to be expected that estimates of the relative contributions of stem reserves to total grain mass per ear or to grain yield would vary among the different reports, according to the experimental conditions and cultivars used. These contributions were estimated to be anywhere between 6% and 100% (Austin et al., 1980; Papakosta & Gagianas, 1991; Pheloung & Siddique, 1991; Davidson & Chevalier, 1992; Borrell et al., 1993; Blum et al., 1994; Gent, 1994; Palta et al., 1994).

It may be concluded that the reduction in current assimilation during grain filling under different stresses will induce greater stem reserve mobilisation to, and utilisation by, the grain. What seems to be important is the reduction in assimilation and not the nature of the stress causing the reduction. Thus, stem reserve mobilisation is a solid source of carbon for grain filling under any stress which would inhibit current photosynthesis, including biotic stresses such as late developing leaf diseases. Tolerance to septoria leaf blotch in wheat is expressed in sustained grain filling under severe epiphytotic. It has been demonstrated that mobilised stem reserve is a major component of septoria tolerance in wheat (Zilberstein et al., 1985).

Drought conditions during grain filling often involve not only water, but also heat stress, which reduces the duration of grain filling. There is normally an increase in the rate of grain dry matter accumulation under high temperatures, but it is not sufficient to compensate for the decrease in duration of filling. When grain filling under such stress depends on remobilise stem reserves, the rate at which these

reserves are metabolised and transported to the grain becomes crucial. It seems that this rate is not sufficiently high to compensate for the reduction in grain filling duration at very high temperatures. Thus, a genetically longer grain filling duration seems to be an advantage in this respect (Blum et al., 1994). Shorter grain filling durations may allow some avoidance of terminal stress while longer duration may allow greater utilisation of stem reserves for grain filling under stress.

### Improving stem reserve utilisation for grain filling

Improving grain filling capacity by stem reserves is an important breeding target in cereals subjected to environmental and biotic stresses during grain filling. Genotypic variation exists for various aspects of grain filling from stem reserves. The effect of height has already been mentioned. With very few exceptions it seems that taller cultivars have a greater capacity to support grain filling from stem reserves because of their greater storage.

The capacity for maintaining large storage in stems appears to be a genetically controlled constitutive trait (e.g., Blum et al., 1994; Hunt, 1979) which may be linked to assimilate partitioning during stem elongation and the developmental characteristics of the stem. If greater stem partitioning is the basis for high reserve storage it might perhaps be at the expense of grain yield potential. Indeed, Pheloung & Siddique (1991) in Australia found that the higher yielding cultivars Gutha and Kulin had less reserve storage and suffered greater reductions in grain yield under drought stress during grain filling, as compared with the potentially lower yielding cultivar Gamenya. This trend was also noted for winter wheat in Kansas, where newer cultivar releases were less capable of grain filling from stem reserves than older cultivars (Hossain et al., 1990). Landraces of wheat were more efficient at grain filling from stem reserves than a modern high yielding cultivar (Blum et al., 1989), though this advantage could also be ascribed to the taller statures of the landraces.

Alternatively, some studies of wheat did not indicate a strong negative relationship between yield

potential and reserve utilisation for grain filling (Blum et al., 1994; Davidson & Birch, 1992). Exceptions were also noted by Hossain et al. (1990), such as the winter wheat cultivar Bounty-310, which had a fairly high yield potential and also good grain filling from stem reserves. However, it remains that cultivars designed for tolerance to stress during grain filling must have the capacity for high stem reserve storage, if necessary even at the expense of a reduction in yield potential.

Delayed monocarpic leaf senescence (syn., non-senescence or stay-green) has long been considered a desirable trait in cereal breeding (e.g., Thomas & Smart, 1993). It is to be expected that longer leaf area duration would contribute to grain filling and yield. However, in two repeated cases for wheat (Blum et al., 1994; Fokar et al., 1998), cultivars of high capacity for stem reserve utilisation for grain filling had accelerated leaf senescence under both stress and non-stress conditions. Inherently accelerated leaf senescence in such cultivars would indicate that stem reserve mobilisation to the grain is a constitutive trait. This seems to be linked to the accelerated export of nitrogen from leaves (e.g., Pell & Dann, 1991). It may therefore be suspected that non-senescence as a sustained source of current assimilation on one hand and stem reserve utilisation on the other may be mutually exclusive. While inherently delayed senescence may be advantageous for yield under optimal growing conditions, it may be of no consequence under post-anthesis stress conditions as the overriding stress factor will impose accelerated senescence or leaf killing. Most likely, selection for non-senescence under non-stress conditions may even prefer genotypes that do not use stem reserves for grain filling.

It seems that large TNC storage is the primary factor for sustaining kernel growth from stem reserves. For example, use of storage for grain filling was found to be proportional to the size of storage across 20 winter wheat cultivars (Hunt, 1979). Also, increased grain filling under stress was proportional to stem sugar concentration at flowering across different wheat cultivars (Nicolas & Turner, 1993). It was noted, however, that some wheat cultivars (e.g., cv. TAM-101) had sufficient storage but were lacking in extent of remobilisation to the grain (Hossain

et al., 1990). The remobilisation and utilisation of the stored carbon depend also on demand. There may be genetic differences among cultivars in enzymatic activity involved with remobilisation, but such activity may also be a function of demand and substrate concentration (Dubois et al., 1990).

Another source of imbalance between storage size and remobilisation is the capacity to deposit starch in the kernel endosperm under heat stress. Soluble starch synthase is a key enzyme in endosperm starch biosynthesis. Compared with all other endosperm starch synthesis pathway enzymes, it is highly thermosensitive, especially at temperatures above 34 °C (Keeling et al., 1993). A more thermostable form (or thermoprotected form) of this enzyme has been identified in a wheat cultivar (Kumar et al., 1998). With this form of heat tolerance in the endosperm, stem reserves were well utilised for kernel growth at temperatures reaching 38-40 °C (Blum et al., 1994). Thus, while the size of the storage is preeminent, the size of the sink and its capacity to utilise the imported carbon is also important for allowing grain filling from stem reserves.

### Methodology and selection

Clarke et al. (1984) demonstrated that simple relationships between stem reserve storage or remobilisation and varietal drought resistance in terms of yield (e.g., by the stress susceptibility index) are not to be expected. Evidently, the impact of stem reserves should be evaluated only under stress conditions that equally inhibit crop assimilation during grain filling in all materials tested.

Selection for better stem reserve supported grain filling under stress may be performed by subjecting the population to the actual stress conditions in the field, such as drought, heat or disease epiphytotic. It has been repeatedly argued that a standard level of biotic or abiotic stress is difficult to achieve during grain filling in diverse genetic materials. The first difficulty is in the techniques for imposing stress on large breeding populations in the field. The second difficulty is in the variable phenology of breeding materials, which does not allow the same timing and degree of stress after flowering in all ma-



terials (Blum et al., 1983b; Mahalakshmi et al., 1994; Clarke et al., 1984).

Blum et al. (1983a, 1983b) proposed the use of chemical desiccation of the canopy after flowering as a means for inhibiting plant photosynthesis and thus revealing the capacity for grain filling by stem reserves. The treatment does not simulate drought stress, but the effect of stress by inhibiting current assimilation. A chemical desiccant (magnesium chlorate or sodium chlorate:  $0.4\% \text{ w v}^{-1}$ ) was applied as a spray to the canopy, including the ears. The treatment was applied to each genotype at 14 days after anthesis, when kernel growth entered its linear phase. At maturity, kernel weight was compared between treated and non-treated (control) plants, calculating the rate of reduction in kernel weight caused by the treatment. The rate of reduction was typically between 5 and 50% in different wheat materials. An important component of this test is that it must be free from any biotic or abiotic stress, so as not to reduce grain filling in the controls (Regan et al., 1993).

Nicolas & Turner (1993) confirmed the utility of chemical desiccation as a means for revealing genetic variation in grain filling from stem reserves and proposed the use of a leaf spray of potassium iodide ( $0.4\% \text{ w v}^{-1}$ ) in wheat as a milder treatment to destroy chlorophyll. Potassium iodide also worked well for millet (Mahalakshmi et al., 1994) and sorghum (Blum, unpublished).

The correlation across diverse genetic materials between the rate of reduction in kernel weight by chemical desiccation and the rate of reduction by drought stress was found to be significant and reasonably high. It was calculated to be  $r = 0.81^{***}$  and  $r = 0.79^{**}$  over two years in Blum (1983b), and  $r = 0.48^{**}$  and  $r = 0.81^{**}$  over two years in Nicolas & Turner (1993). The relationship also held for several millet genotypes treated with KI (Mahalakshmi et al., 1984). Hossain et al. (1990) noted that winter wheat cultivars of stable kernel weight over years and locations sustained relatively less reduction in kernel weight under sodium chlorate desiccation of the canopy. Blum et al. (1994) found a correlation of  $r = 0.94^{**}$  across five wheat cultivars between the reduction in kernel weight by chemical desiccation and the reduction in kernel weight by heat stress

(35/25 °C, day/night temperatures) during grain filling. The rate of reduction in kernel weight under harsher heat stress conditions was well correlated across different wheat cultivars ( $r = 0.74^{**}$ ) with kernel weight reduction caused by post-anthesis defoliation and shading of plants under optimal temperatures (Fokar et al., 1998). Finally, the reduction in kernel weight by chemical desiccation was significantly correlated across different wheat cultivars ( $r = 0.48^{*}$ ) with reduction in kernel weight caused by late epiphytotic of septoria leaf blotch disease (Zilberstein et al., 1985).

Chemical desiccation can be incorporated into breeding programs in two ways. Firstly, it can be used to assess responses of individual advanced lines or families, always compared with non-treated controls under non-stress conditions. This can be easily performed with nursery ear-rows. Secondly, it can be used in mass selection. Blum et al. (1991) performed mass selection, where six spring wheat  $F_2$  bulks were chemically desiccated with magnesium chlorate, after which grain were divergently selected for kernel weight by mechanical sieving. After two or three cycles of selection, random lines were selected and tested for their response to chemical desiccation stress. Mass selection for large kernels under chemical desiccation significantly improved kernel weight and grain yield under chemical desiccation stress, as compared with controls where selection for kernel weight was performed without chemical desiccation. There was no shift in phenology or plant height under chemical desiccation selection, probably because the variation in these traits within the populations used was small.

Haley & Quick (1993) performed a similar selection program under chemical desiccation with sodium chlorate in winter wheat. Two cycles of selection produced  $F_2$  bulks that were more resistant to chemical desiccation stress.

## Conclusions

Stem reserves offer a powerful resource for grain filling under any type of stress which inhibits current assimilation. In 1983, chemical desiccation was proposed as a method of selection for improved

grain filling from stem reserves. Since then the method has been confirmed by several independent studies to be useful and effective.

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