Seed growth rate in grain legumes
II. Seed growth rate depends on cotyledon cell number

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Abstract

Individual seed weight and seed growth rate are variable within the plant and among environmental conditions. Seed growth rate remains constant during the filling period even if assimilate availability is modified. This paper describes the relationship between the cotyledon cell number fixed at the beginning of seed filling and the seed growth rate. Two genotypes of pea were grown in various environmental conditions: field, glasshouse and growth chamber. One genotype of soybean was sown in field. Seed growth rate and cotyledon cell number were measured. Variations in seed growth rate (0.24 to 1.07 mg per degree-day for pea, 0.23 to 0.42 mg per degree-day for soybean) largely account for differences in individual seed weight. For each species, cotyledon cell number (from 3.4 × 10⁵ to 10.2 × 10⁵ per seed for pea, from 6.7 × 10⁶ to 9 × 10⁶ per seed for soybean) and seed growth rate are strongly correlated regardless of environmental conditions and intraplant position. Consequently, seed growth rate observed during the seed filling period is determined before this period during the cell division in the embryo: variations in seed growth rate depend on the growing conditions during the period between flowering and the beginning of seed filling.

Key words: Grain legume, seed growth rate, cotyledon cell number.

Introduction

Yield variability in pea (Pisum sativum L.) (Hardwick, 1988) and of soybean (Glycine max L. Merr) (Shibles et al., 1975) in various environmental situations is mainly correlated with changes in seed number. However, variations in individual seed weight can still account for differences in yield between environments for one genotype, as well as between genotypes in one environment. Individual seed weight is commonly analysed as the product of seed growth rate by duration of seed filling. Genotypic differences in seed weight are mainly correlated with differences in seed growth rate (Egli et al., 1981). In the companion paper, Munier-Jolain et al. (1998) have shown for three grain legume species (lupin (Lupinus albus L.), pea, and soybean) that changes in photoassimilate availability do not affect seed growth rate if treatments are applied after the completion of embryo cell division. A reduction in assimilate availability does not lead to a decrease in seed growth rate, whereas total plant growth and duration of seed filling are reduced. In the same way, an increase in assimilate availability does not cause any increase in seed growth rate. Seeds have effectively often been described as a high priority sink in assimilate partitioning (Minchin and Thorpe, 1996). Thus, assimilate flux in growing seeds fluctuates little and it can be assumed that seed growth rate remains constant during the seed filling period. Consequently, the seed growth rate observed at the beginning of seed filling corresponds to a potential seed growth, which should be determined before the period of rapid seed growth.

Seed development and growth can be divided into three phases (Munier-Jolain et al., 1993). The first one begins at flowering and ends at the beginning of seed filling. During this period, cell division occurs in the embryos without significant dry matter accumulation (Ney et al., 1993, on pea; Egli et al., 1981, on soybean). At the end of this phase, cell division stops, linear dry matter accumulation begins in cotyledons and continues until physiological maturity. Some authors have proposed for many species that seed cell number could account for the genotypic variability in seed growth rate (Guldan and

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Materials and methods

Plant material and growing conditions

Experiments were conducted with two grain legume species: pea and soybean.

Pea

Cultivars ‘Solara’ and ‘Frisson’ were sown in 3 replicates of 6 row plots at 80 plants m\(^{-2}\) with a 20 cm row-spacing at INRA experimental station in Dijon (France) on 6 March, 10 March, 2 March, and 4 June 1992 and on 10 March 1995 and 4 March 1996, and at INRA experimental station in Mons (France) on 18 March and 18 April 1991, and on 3 March 1992. Experiments were well irrigated and received a nutrient supply (P and K) to avoid moisture or nutrient stresses. *Rhizobium leguminosarum* was present in the soils. For all field experiments except in 1995, seeds from 10 plants per replicate of the three most productive nodes of each genotype were harvested separately twice a week until maturity, and were bulked. For the field experiment in 1995, data were recorded for the two lowest reproductive nodes. Solara and Frisson were sown in a growth chamber on 16 December 1993, with 4 plants per pot, under a 13 h photoperiod. Nutrient solution (P and K) was provided by regular watering of pots. *Rhizobium leguminosarum* was inoculated at sowing. The beginning of flowering was recorded on each plant. After the beginning of flowering, all reproductive nodes except the first one were deflowered to maintain a high source–sink ratio, so that the remaining seeds could reach their maximum seed growth without any limitation of assimilate supply. One pod at the first node of all plants of five pots was harvested every 2 d.

Soybean

An indeterminate maturity group 00 soybean cultivar ‘Maple Arrow’ was sown on 5 June 1991 and 31 January 1992 at the INRA experimental station of Guadeloupe (French West Indies) in four replicates. Plant densities were 20 and 80 plants m\(^{-2}\) in 1991 and 20 plants m\(^{-2}\) in 1992 (with 30 cm row-spacing). Experiments were well irrigated and received a nutrient supply (P and K) in order to avoid moisture or nutrient stresses. *Bradyrhizobium japonicum* was inoculated at sowing. Ten plants per replicate were collected twice a week during seed filling period. On the mainstem (i) seeds at each morphological position (primary and secondary racemes on each node of the mainstem) for the low density in 1991, and (ii) seeds from the second and fifth node for high density in 1991 and in 1992, were bulked. Primary and secondary racemes at each reproductive node did not flower simultaneously and thus were collected separately. Secondary racemes did not produce any pod in the high density situation. Seeds from branches were not collected.

Measurements

For both species, for each experiment, at each sampling date, seeds were counted, fresh weight (FW) and dry weight (DW) after oven drying at 85°C for 48 h were measured to calculate the mean individual seed mass and the seed water concentration (WC) as

\[
WC = \frac{FW - DW}{FW}
\]

Dry weight per seed was plotted versus cumulative degree-days from sowing. Seed growth rates were assessed as the slopes by linear regression on seed weight versus thermal time during the filling period defined by seed water concentration (Munier-Jolain et al., 1993; Dumoulin et al., 1994).

At harvest, the testas of all sampled seeds were removed: individual seed weight and testa weight were measured after oven drying at 85°C for 48 h. For each experiment, testas were removed from 10 seeds per replicate from each seed group at maturity and cotyledon cell number was measured with the method proposed by Brown and Rickless (1949): the seeds were macerated and digested in a chromic acid solution (50 g CrO\(_3\)·H\(_2\)O l\(^{-1}\)) for 16 h. The cell number was counted using a hemacytometer slide under a microscope at a magnification of 100\(\times\).

Time was expressed as degree-days cumulated from sowing with a 0°C base temperature for pea (Ney and Turc, 1993), a 6°C base temperature for soybean (Munier-Jolain et al., 1993). Statistical analysis were performed with GLM procedure of SAS (SAS Institute, 1987).

Results

During the first period between flowering and the beginning of seed filling, seed water concentration was constant at approximately 0.85 g g\(^{-1}\) (Fig. 1), as already shown in pea by Ney et al. (1993), and in soybean by Munier-Jolain et al. (1993). When the linear phase of dry matter accumulation began, different percentages of the final seed weight had already accumulated (Fig. 1): approximately 10% for both cultivars of pea, and less than 8% for soybean. At maturity, testa weight represented 10% and 8% of the final seed weight for pea genotypes Solara and Frisson, respectively, and 7% of the final seed weight for soybean. Physiological maturity was reached when the seed water concentration decreased under 0.55 g g\(^{-1}\) for pea (Ney et al., 1993) and 0.60 g g\(^{-1}\) for soybean (Munier-Jolain et al., 1993). Dry matter accumulation in the seeds stopped because the vascular connections between the seeds and plant were disrupted.

The average weight per seed at harvest was highly variable among species, genotypes, growing conditions and nodal positions (Fig. 2). For pea, the average weight per seed varied significantly, from 99 to 389 mg per seed for Solara and from 69 to 224 mg per seed for Frisson.
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Fig. 1. Individual seed weight (■) and seed water concentration (□) versus time. Vertical lines on the x-axis indicate the estimated time of the beginning of seed filling.

(Fig. 2). For both genotypes the minimum and maximum values of average weight per seed corresponded respectively to the latest sowing date in the field (June) and to a winter sowing date in the glasshouse for depodded plants. The mean individual seed weights across different environmental conditions for Solara and Frisson were 277 and 138 mg per seed, respectively.

For soybean average weight per seed at harvest varied significantly from 111 at 20 plants m$^{-2}$ in 1991 to 225 mg per seed at 20 plants m$^{-2}$ in 1992 (Fig. 2). Average seed weight also varied among morphological positions. For example, in Guadeloupe 1991 at the lowest density, seeds from the primary racemes were always larger than those from the secondary racemes (Table 1).

For both species, variations in average weight per seed among experimental situations were highly correlated with differences in individual seed growth rate (Fig. 2): the largest seeds had the highest seed growth rates.

Cotyledon cell numbers were highly variable (Fig. 3).

(i) For a given genotype across environmental situations,
1974 Munier-Jolain and Ney

Discussion

For several species, the final individual seed weight is largely correlated to the seed growth rate (Brocklehurst, 1977; Egli et al., 1981; Guldan and Brun, 1985; Sexton et al., 1997), whatever the cause of variation in seed growth rate (genotype, environment, nodal position). Therefore, the analysis of variation in seed growth rate is useful for trying to understand the variation in individual seed weight.

According to Ney et al. (1993) and Munier-Jolain et al. (1993), dry matter accumulation in the seed becomes appreciable when seed water begins to decrease below 0.85 g g\(^{-1}\). The percentage of testa weight in the seed weight at maturity for both species was similar to the percentage of seed weight already accumulated at the beginning of seed filling. Hedley et al. (1994) have shown that growth of the testa approximately ends when the active filling period begins. Consequently, at the beginning of the seed filling, the individual seed weight corresponds to the testa weight. Variations in seed weight at the beginning of seed filling according to the legume species seems to be due to variations in the percentage of testa weight in the final seed weight among species (20% for lupin, Munier-Jolain, unpublished data). Consequently, the seed growth rate corresponding to the accumulation of carbohydrates reserves in the seed should be calculated

Fig. 3. The relationship between cotyledon cell number and seed growth rate. Pea: Frisson (■) and Solara (□), in various environmental conditions. Circles denote seeds from depodded plants. Soybean: 1991, 20 plants m\(^{-2}\) (●), 1991, 80 plants m\(^{-2}\) (○), 1992, 20 plants m\(^{-2}\) (■); for each symbol, different points correspond to different morphological positions within the plant.

cotyledon cell number varied from 0.34 \(\times\) 10\(^6\) to 0.9 \(\times\) 10\(^6\) for Frisson, from 0.56 \(\times\) 10\(^6\) to 1.02 \(\times\) 10\(^6\) for Solara (Fig. 3), and from 6.72 \(\times\) 10\(^6\) to 9.0 \(\times\) 10\(^6\) for soybean (Fig. 3): the later the sowing date, the smaller the seed cell number; (ii) between genotypes the mean values of cell number were 0.61 \(\times\) 10\(^6\) and 0.84 \(\times\) 10\(^6\) for Frisson and Solara, respectively; (iii) between morphological position on soybean, cotyledon cell number varied from 6.72 \(\times\) 10\(^6\) (\(±\) 0.23 \(\times\) 10\(^5\)) to 8.39 \(\times\) 10\(^6\) (\(±\) 0.24 \(\times\) 10\(^5\)) in 1991 at the low plant density.

Whatever the species, the genotypes or the environmental conditions, cotyledon cell numbers and seed growth rates were closely related (Fig. 3): the greater the cotyledon cell number, the higher the seed growth rate. For Solara and Frisson, cotyledon cell number and seed growth rate of seeds collected on depodded plants in the glasshouse were consistent with the relationship established with seeds collected in field conditions on well-podded plants (Fig. 3).

Relationship between cell number and growth rate

For a given genotype, differences in seed growth rate among morphological positions on the same plant (soybean) and among environmental conditions, are strongly and positively correlated with differences in seed cell number. In the same way Egli et al. (1989) on soybean and Brocklehurst (1977) on wheat have shown that changes in seed cell number produced by changes in assimilate availability during cell division led to changes in seed growth rate.

The relationship between seed growth rate and cotyledon cell number is valid across genotypes characterized by different seed size. For several legume species or cereals, Brocklehurst (1977) on wheat, Guldan and Brun (1985) on soybean, and Sexton et al. (1997) on common bean (*Phaseolus vulgaris* L.), have shown that seed weight differences among genotypes are mainly due to differences in seed cell number, suggesting that the seed cell number...
is a major factor controlling the seed growth rate and the final seed weight.

For pea, seeds collected on depodded plants had cotyledon cell numbers and seed growth rates consistent with the relationship obtained in field conditions. As seeds of this experiment were harvested on plants which were still photosynthetically active, assimilate availability probably did not limit growth so that the potential seed growth rate could be reached. Therefore, seed cell number fixed at the beginning of seed filling can be a strong determinant of the seed growth rate. This result is consistent with that of Munier-Jolain et al. (1998) which showed that the deposition of storage compounds in filling seeds constitutes a strong priority for assimilates whatever the assimilate availability.

A limiting source of assimilates during the seed filling period does not affect seed growth rate, but modulates the duration of seed filling as reported by Munier-Jolain et al. (1998) on soybean or Jones and Simmons (1983) on maize (Zea mays L.). For several legume species or cereals, cell division in seeds is completed when the seeds enter the linear phase of growth which corresponds to the period of storage accumulation (Egli et al., 1981, on soybean; Ney et al., 1993, on pea; Brocklehurst, 1977, on wheat; Jones et al., 1996, on maize). For legumes, the final cell number of the embryo is reached early in its development and its subsequent increase in mass is the result of cell expansion and the concomitant deposition of carbohydrates and nitrogen reserves (Dure, 1975). Consequently, the cotyledon cell number at the end of cell division cannot be a consequence of the seed growth rate during seed filling which occurs later, but seems to be a good predictor of subsequent seed growth: if cell number is low, seed filling rate cannot be increased even if assimilate sources are large (Munier-Jolain et al., 1998).

Jones and Simmons (1983) also suggested that the sink capacity could be a growth-limiting factor of yield on maize. This interpretation of the relationship between cell number and growth rate is not in accordance with the hypothesis recently suggested and argued about cell division and expansion. Results on the expansion of sunflower leaves (Helianthus annuus L.) (Granier and Tardieu, 1998) and on stem elongation in deepwater rice (Oryza sativa) (Jacobs, 1997) gave convincing arguments in favour of the theory of cell division triggered by cell expansion to ensure cell size homeostasis. However, those results were obtained for plant organs which differed notably from filling seeds as (i) they did not accumulate storage compounds while elongating and (ii) their elongation and growth occurred with concomitant cell division.

Variability in seed cell number

On several species, differences in seed size among genotypes are significantly correlated with cotyledon cell number (Brocklehurst, 1977; Cochrane and Duffus, 1983; Egli et al., 1981; Guldan and Brun, 1985): in these experiments, the seed cell numbers of the cultivars Frisson and Solara, respectively, small- and large-seeded genotypes, are in accordance with this trend. On maize (Jones et al., 1996), and on pea (Déjardin-Séry, 1995), a comparison of seeds from reciprocal crosses of lines which differed in seed size, revealed a strong maternal influence on seed mass. Those authors showed that the maternal effect on seed size was due to changes in cell number.

In spite of the strong genotypical determinism of seed cell number, a given genotype exhibits differences in seed cell number among environmental conditions (Swank et al., 1987). The results of these experiments confirm this effect of environmental conditions on cell number, as late sowings in spring led to low cotyledon cell numbers. Several authors have shown changes in cell number induced by a changed source–sink ratio during cell division. An increase in assimilate availability during the cell division period by fruit removal causes an increase in the final seed cell number (Brocklehurst, 1977; Egli et al., 1989) and a decrease in assimilate availability by shading or defoliating during the cell division period causes a reduction in final seed cell number (Egli et al., 1989; Singh and Jenner, 1984). For the experiment in 1995 in the glasshouse, a depodding treatment applied during the period of cell division triggered an increase in cotyledon cell number for seeds on depodded plants as compared to control plants. In the same way, cell division for in vitro-cultured embryos increases with increasing sucrose concentration (Singh and Jenner, 1984). Cell division could probably be affected by the amount and quality of assimilates supplied to seeds during the lag-phase before the beginning of seed filling, in accordance with the theory of control by cell size.

Moreover cell division is influenced by environmental conditions as temperature (Egli and Wardlaw, 1980; Jones et al., 1984; Wardlaw, 1970) or water stress (Ouattara et al., 1987). Extreme temperatures applied during cell division cause a reduction in cell number of in vitro-cultured embryos, whereas all treatments (extreme temperatures and control) were grown with the same sucrose concentration (Jones et al., 1984). Consequently, extreme temperatures can affect cell division per se regardless of the assimilate availability. Thus cell division appears to be controlled by genetic, assimilate supply and environmental conditions during cell division period.

Conclusion

For both grain legume species, pea and soybean, differences in seed growth rate for the same genotype among environmental conditions or among pod positions within the plant, and among genotypes, are linked to differences in seed cell number. Since seed growth rate is not affected...
by assimilate availability, the determination of seed potential growth rate must be analysed during the cell division period, between flowering and the beginning of the accumulation of storage compounds. Seed cell number depends on genotype and on environmental conditions during cell division.

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References