GENETIC AND ONTOGENETIC VARIATION FOR STORAGE PRODUCT COMPOSITION IN DIVERSE ACCESSIONS OF *BRASSICA JUNCEA*.

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ABSTRACT

Seed from 35 genotypes of field grown *Brassica juncea* were analysed for lipid and protein content both of which varied from 28 to 54% of embryo weight. Lipid content was negatively associated with protein content and with date of flowering while, interestingly, protein content was positively correlated with height.

INTRODUCTION

Plant storage organs synthesise proteins, starch or oils and considerable variation in the partitioning among these storage compounds is found. Starch is the main storage product in cereals while both protein and starch are found in legumes. In oilseeds however, (*e.g.* soybean, palm, sunflower and rape) lipids are the principal storage compounds, typically accounting for between 40-50% of seed weight with smaller amounts of protein, typically 20-30%. Starch is only a minor component though it does accumulate as a transient product during the development of the embryo (P. da Silva and A. M. Smith: pers. comm.). We are interested in understanding the biochemical mechanisms involved in this partitioning and its control. The pathways involved, however, are complex and it will be important to have isogenic lines with different patterns of storage product accumulation for investigations into these mechanisms.

Brassica napus has undergone strong selection pressure for oil content and quality and is now the most important of the oilseed crops grown in the temperate climatic zones of the world. This means that within *B. napus* there is only limited variability in oil content. In contrast *B. juncea* has been grown variously as an oil, mustard or vegetable crop over a wider geographical range and environmental conditions and there is much morphological variation among mustard cultivars (Vaughan and Hemingway, 1959). Greater genetic diversity is likely among these cultivars because of the different selection pressures put on them *i.e.* for high oil content, suitability as a condiment and for the vegetative form (no selection for oil or protein) respectively. Additionally, *B.napus* is thought to have arisen in a single place whereas *B. juncea* is believed to have come from two main centres of origin, India and Pakistan and China. These factors make it likely that there will be a greater range of genetic diversity in *B. juncea* than *B. napus* which can be used in producing isogenic lines with large differences in storage product accumulation. The work described here characterises such variation among a set of *B. juncea* cultivars.

EXPERIMENTAL

Thirty five genotypes differing widely in origin and use were sown in John Innes no.1 potting compost and grown in a glasshouse at 20/10 °C day/night during March 1993. Eleven of these genotypes were yellow-seeded while the rest were either black- or brown-

seeded. Five plants of each genotype were transplanted into the field 50 cm apart in rows 1 m wide when they had reached the fourth leaf. Measurments of date of flowering, mean seed weight and height were made on the three central plants for each genotype. One plant was bagged to obtain selfed seed for the determination of lipid, protein and starch composition.

Total lipids were determined by quantitative fatty acid methyl ester analysis (Kang *et al.*, 1993) of three replicate samples of between 15-20 mg mature seeds (4% moisture). Total proteins were estimated by macerating 15-20 mg mature seeds in a pestle and mortar using a grinding medium containing 0.4 M sucrose, 50 mM tris-HCl, 1 mM EDTA and 1% SDS. The samples were centrifuged at 4000*g* for 5 min and then diluted with water to an appropriate concentration before protein determination. Proteins were determined using Bicinchoninic acid and measured at 563 nm on a micro-titre plate reader using bovine serum album standards for the calibration.

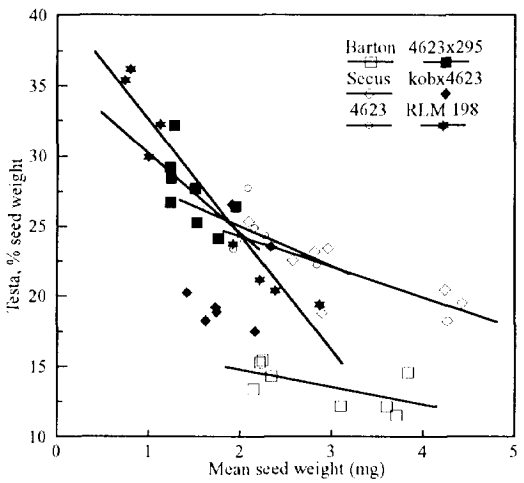

Figure 1. Testa dry weight (% seed weight) in selected genotypes of *B. juncea*.

Because the testa weight was likely to represent a variable proportion of the seed weight amongst the genotypes (Abraham and Bhatia, 1986; Stringham *et. al.*, 1974) we wished to express the storage product composition on an embryo basis. The testa of *B. juncea* seed adheres tightly to the embryo and is impossible to remove when the seed is dry. Because of this testa dry weights were determined by imbibing a sub-sample of five pre-weighed seeds in water overnight and then removing the testa from the swollen seeds. Eight samples of each cultivar with different mean seed weights were used. These were then dried at 80 °C, weighed and the results expressed as a percentage of seed dry weight (Fig. 1). Lighter seeds have a higher testa : embryo ratio than heavier seeds. Yellow-seeded genotypes had on average 4% less testa than the brown/black seeded genotypes despite having a 20% greater mean seed weight (18 and 22% respectively). There were significant differences among the genotypes within each colour group which were independent of seed size.

High lipid or protein content was associated with low protein or lipid content respectively, particularly at the extremes of the distribution, though the overall correlation was less strong ($r=-0.465^{**}$) which accounts for only 22% of the total variation. One possible reason for this variation is that the sum of the lipid and protein does not account for all the embryo weight. The remainder is attributable to cell walls, prescence of uncharacterised storage compounds within the embryo such as α-galactosides and smaller amounts of soluble sugars. Initial analyses showed that, similarly to *B. napus*, only small amounts of starch are present in the mature seed.

TABLE 1. Lipid and protein contents of embryos from seven *B. juncea* genotypes and other phenotypic characters

Genotype	Date of flowering	Height (cm)	Mean seed wt.	Testa (% seed wt.)	Protein (% embryo wt.)	Lipid (% embryo wt.)	Total lipid + protein
Barton	17 May	129	2.13	14.1	36.8	45.4	82.3
Secus	23 May	138	3.85	22.0	43.1	30.9	74.0
34285	7 June	127	3.65	25.4	53.5	29.7	83.2
4623	14 May	148	2.32	22.7	33.2	54.3	87.4
4623x295	*17 May*	*77*	*1.50*	*28.3*	*35.9*	*43.5*	79.4
Kobx4623	9 May	34	1.56	21.9	42.6	48.0	90.6
RLM 198	2 June	125	1.81	29.4	48.2	34.7	82.9
SE	-	10.1	0.26	-	1.46	3.39	3.69

Results for seven selected genotypes chosen to represent high lipid, high protein and high total storage material are summarised in Table 1. Plant height was positively correlated with protein content ($r=0.497^{***}$) and negatively with lipid content ($r=-0.359^{**}$). It is not known what the reason for this is. There was a negative correlation (-0.426) between lipid content and date of flowering which may either reflect different rates of maturation or differences in the environmental conditions experienced by the plant during seed filling.

This work has been repeated during 1994 together with further experiments to investigate different rates of storage product accumulation among selected genotypes.

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