Seed development and oil accumulation in canola, *Brassica napus*.

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ABSTRACT

To alter the pattern of seed growth and maturation, lateral inflorescences were removed restricting flowering to the main axis. This treatment manipulated the source sink ratio in three cultivars that show a range of oil concentrations in the field. We expected increased growth of the cotyledons would lead to an increase in oil concentration in the seed. Plants were grown in containers in a sunlit glasshouse at 20/13˚C.

Pods reached maximum size 10 daf. The pruning treatment did not affect pod length in cvs Monty and Mystic (78mm) but increased pod length in Karoo from 58 to 64mm. Ovule number per pod was 21 to 23 and was unaffected by pruning in all cvs. Pruning increased pod cross sectional area in Monty by 30% and Mystic by 25% but not in Karoo. In Monty it increased time to 50% embryo de-greening by 3 days and to 50% black seed by 2 days.

Ovule growth peaked 30 daf and pruning increased ovule size by 12 to 21%. Embryo growth peaked at 35 to 40 daf and then remained steady till maturation. Initially, the ovules were significantly larger than the embryos but they shrank as the seed began to dry, finally fitting snugly around the embryo. Thus the embryo was not limited by space during its period of rapid expansion. Pruning increased whole embryo area in Monty and Mystic by 22% but had no significant effect in Karoo.

At maturity control seeds were heavier than parent field gown seeds by 30 to 50%. The oil concentration was unchanged, despite the larger seed size. Pruning decreased oil concentration by 2 to 3%. Thus the increase in seed size, caused by changing the source sink ratio, was slightly greater than the concomitant rise in oil content per seed.

Key words: Ovule – seed – oil – embryo – cotyledon.

INTRODUCTION

In the seed of *Brassica* sp. to 90% of the oil is found in the cotyledons. Oil accumulation depends on the supply of sugars to the growing seed and the capacity of the cotyledons to store oil. Seed oil concentration is therefore influenced by the cotyledon size relative to the whole seed and oil concentration in the cotyledon. Therefore knowledge of their development will impact on oil accumulation. Increasing cotyledon size could be expected to increase seed oil concentration. Thus seed size could provide a useful selection criterion for crop improvement.

Removing axillary inflorescences reduces competition between individual flowers for carbon compounds imported from leaves and stems. This manipulation of the source sink ratio is likely to produce a physiological response that would alter the normal pattern of embryo growth and maturation and could be expected to increase oil concentration. Parameters of pod and embryo growth and oil content were examined to determine if reduced competition for resources caused a physiological change in the reproductive structures and embryo that would lead to increased oil deposition.

MATERIALS AND METHODS

Cultivars chosen were Monty, Mystic and Karoo which give an oil concentration gradient. Plants were pot grown in a sunlit phytotron, at day/night temperatures of 20/13ºC. The lateral inflorescences were removed as they appeared. Unpruned plants were controls. Plants were open pollinated. Each flower was labelled at anthesis giving a population of pods of known ages. Pods, ovules and embryos were harvested at 2 day intervals from 10 daf to maturity. Samples were fixed in 2.5% glutaraldehyde in phosphate buffer. Measurements consisted of pod length, and pod area in cross section. Ovules were removed and area, length and width measured. Embryos were excised and laid flat to measure area, length and width of the embryo and its organs, the cotyledons and radicle. Oil concentration was measured in the mature seed by the Chemistry Centre, WA.

RESULTS

Oil accumulation

The control plants produced seeds that were 38% larger than the parent field grown seeds but they had the same oil concentration. Pruning increased seed weight another 24% but decreased oil concentration by 2% (Table 1). The increase in seed size was greater than the concomitant increase in oil content, leading to a concentration decline.

Table 1. Seed weight and oil accumulation in mature seed of parent, control and pruned plants.

	Seed weight (mg/seed)			Oil concentration (%)		
Cultivar	Parent	Control	Pruned	Parent	Control	Pruned
Monty	3.10	4.20	5.50	44.90	45.05	42.50
Mystic	3.30	4.30	5.45	45.60	42.65	42.25
Karoo	3.00	4.50	5.15	43.20	42.15	39.80
Mean	3.13	4.33	5.37	44.57	43.28	41.52
se	0.09	0.09	0.11	0.71	0.90	0.86

Pod growth

The pods reached maximum length 10 daf. Pruning had little effect on the length of pods of Monty and Mystic but increased length in Karoo by 10% from 58 to 64mm. Pod cross sectional area peaked 25 to 35 daf, declining slightly towards maturation. Pruning increased pod area 20 to 40% without increasing pod wall thickness.

Ovules

Pruning had little effect on the number of ovules per pod (22 to 23). It increased the time to 50% seed blackening in Monty by 2 days, from 42 to 44 daf and in Karoo by 1 day, from 44 to 45 daf. Ovule growth peaked at 30 daf and declined slightly towards seed maturation. Pruning increased ovule size in Monty by 15% from 4.5 to 5.2mm^2, in Mystic by 21% from 4.3 to 5.2mm^2 and in Karoo by 12% from 4.5 to 5.0mm^2.

Table 2. The effect of pruning lateral inflorescences on the maximum size of tissues in the embyros of three cultivars of canola. S= significant and NS= not significant at P=0.05.

		Maximum growth (mm^2)					
Organ	Cultivar	Pruned	se	Control	se	Diff	P
Whole embryo	Monty	14.9		12.3		+21%	S
	Mystic	14.8		12.0		+23%	S
	Karoo	13.6		14.5		-6%	NS
Outer cotyledon	Monty	7.4	0.2	6.0	0.2	+23%	S
	Mystic	7.2	0.2	5.7	0.2	+26%	S
	Karoo	6.5	0.3	6.8	0.3	-4%	NS
Inner cotyledon	Monty	6.2	0.2	5.2	0.2	+20%	S
	Mystic	6.2	0.3	5.0	0.2	+23%	S
	Karoo	5.7	0.2	6.1	0.3	-6%	NS
Radicle	Monty	1.3	0.02	1.1	0.03	+15%	S
	Mystic	1.4	0.07	1.3	0.05	+8%	NS
	Karoo	1.4	0.07	1.5	0.06	-1%	NS

Embryos
Embryo growth peaked around 40 daf. Pruning increased the time to 50% de-greening in Monty by 3 days from 44 to 47 daf. It increased whole embryo area in Monty by 21%, Mystic by 23% but had no significant effect in Karoo (Table 2). Pruning increased the outer cotyledon area in Monty by 23%, Mystic by 26% and had no significant effect in Karoo. It increased the inner cotyledon area of Monty by 20%, Mystic by 23% but had no effect in Karoo. In addition, pruning increased the radicle area of Monty by 15%, Mystic by 8% and had no effect in Karoo. Pruning increased the size of the outer cotyledon about 3 to 5% more than the inner cotyledon in Monty and Mystic, but had no significant effect in Karoo.

DISCUSSION

Removal of axillary inflorescences increased the embryo size, especially the outer cotyledon in Monty and Mystic, Karoo was relatively unaffected by pruning in most parameters, except increased pod length. Pruning enlarged the cotyledons of Mystic the most and increased the radicle the least, compared with Monty, but it also reduced oil concentration more. Overall Monty benefited the most with a good enlargement of cotyledons especially the outer cotyledon with a moderate reduction in oil concentration. The larger seeds contained more oil per seed, but a slightly reduced oil concentration. Therefore, increasing the supply of carbohydrates to the seed, by changing the source sink ratio, has increased the size of all seed components proportionately. In this case, increased seed size did not result in increased oil concentration, which is of commercial interest.

Yield of canola is much more strongly influenced by seed number per unit area than by seed size (Hocking *et al.* 1997). Thus, observed positive associations between yield and oil concentration are likely to be the consequence of factors, that may be auto-correlated, and acting independently on yield (eg water supply) and oil concentration (eg temperature).

In this experiment, reduced competition for resources at the critical stage of embryo development resulted in significantly larger ovules, seeds and embryos. Reducing competition between individual flowers early in development increased individual seed weight especially the embryo and its outer cotyledon, without increasing seed number per pod.

Pruned plants continued to produce axillary inflorescences which, although they had their flowers removed, failed to de-leaf or senesce at seed maturity but continued growing. Fatty acid biosynthesis requires carbon skeletons and energy (O'Hara *et al.* 2001). The new leaves on lateral inflorescences may have been significant sinks because they failed to senesce in the pruned treatments. These small leaves may have competed with growing reproductive parts on the main stem, for carbon, especially late in seed development. This may be associated with the failure to accumulate more oil in the cotyledons.

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