TOTAL GLUCOSINOLATES, GLUCOSE AND MYROSINASE ACTIVITY DURING RAPESEED RIPENING(*Brassica napus* L.var.Oleifera)

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Quantitative and qualitative yields of the majority of agricultural crops depend on the degree and trend of ripening. In the case of the rape plant the nutritional and technological quality of the seed and the defatted meal in particular are greatly affected by the glucosinolate content (Astwood et al. 1949). It is known that these compounds, when hydrolyzed by myrosinase, yield toxic substances (Van Etten and Wolff, 1973) that can also react with the proteins in the meals,thereby decreasing the nutritional value considerably (Kawakishi and Kaneko, 1987). For this reason, one breeding objective in recent years has been the creation of high-yield varieties without glucosinolates, or with only small quantities.

While it is generally accepted that the common precursor of glucosinolates is methionine (Chisholm and Wetter, 1967), their concentrations depend not only on genetic factors, but also, for a given genotype, on the stage of seed development (Kondra and Downey, 1969). It has been reported that the total glucosinolate content also depends on nitrogen nutrition (Josefsson, 1970). In this regard it should be mentioned that in five years of tests with numerous samples from different Italian environments we found no correlation between the parameters considered (Leoni et al. 1985).

This paper is part of a series of studies on the quality of cruciferous oil-bearing seeds with special reference to glucosinolates and myrosinase (Palmieri et al. 1982; Iori et al. 1983; Palmieri et al. 1986). The results of these studies have induced us to focus our attention on the trend of these compounds during seed maturation.

Our tests were designed with two main objectives: (i) to reveal the trend of some quantitative and qualitative factors of maturing seeds, viz. the relative increase in dry matter, oil, protein, glucose, glucosinolates, and myrosinase activity and (ii) to determine if there is a correlation between glucosinolate content and myrosinase activity in specific stages of seed ripening. With regard to the second aim, which should give us a better understanding of the physiology of seed ripening with an eye towards genetic improvement, the results of our previous tests conducted on numerous samples of completely ripened seed were uncertain (Palmieri, 1983).

Our experiments were agronomic variety-comparison tests conducted in 1986 on seed samples collected at 3-4 day intervals starting about 10 days after the completion of anthesis (ca. 90% flower bulk) of three winter rape varieties: Jet Neuf (type 0), Lingot (type 0 with average glucosinolate content) and Jade (type 00). Because the first-harvest seeds (up to 10th, 13th and 16th days for the Jade, Lingot and Jet Neuf cv. respectively) were too small and had a humidity content that was too high, it was not possible to make up a sample suitable for the planned analyses.

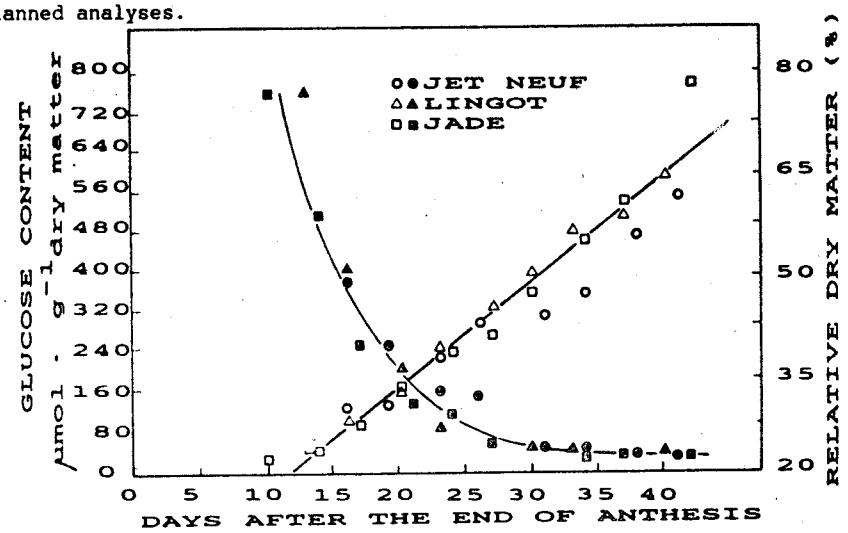

Fig. 1. Glucose uptake (●▲■) and increase in relative dry matter (○△□) during seed ripening.

Fig.1 shows the relative increase in dry matter and glucose consumption during seed ripening. Although these results are preliminary and require further investigation, the trend of these parameters and their crossing point should serve as an objective reference for establishing the effect of environment on yield, thus permitting its estimation. Although the genotypes differed in more than one respect, the trends of the two parameters do not show appreciable differences, with the exception of the Jet Neuf cv., which slowed its relative increase in dry matter after the 25th day, mainly with regard to oil content, as can be seen from table 1.

Fig.2 shows the trend of glucosinolate content for the three cultivars, which are known to differ in the genetic expression of this trait. Jade, in fact, while beginning to synthesize glucosinolates in the early stages stayed within 18 µmoles/g of dry matter, whereas Lingot, after a rapid increase up to approx. 50, leveled off at approx. 60 µmoles/g. Jet Neuf showed a delayed synthesis of glucosinolates, followed a rapid and prolonged increase of these compounds, reaching a maximum content in the final stages or even in the completely ripened seed, ready for processing. The trends for these three genotypes make it reasonable to assume the existence of a regulating mechanism not only for the synthesis of glucosinolates, but also for their demolition.

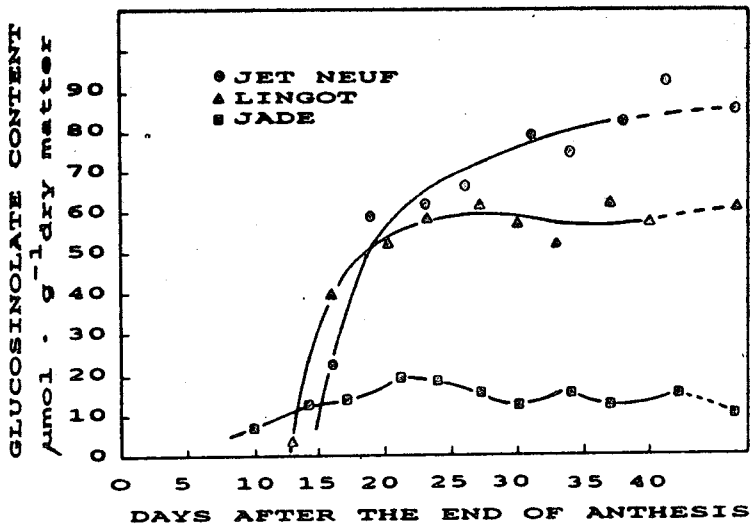

Fig. 2. Glucosinolate content during seed ripening.

TABLE. 1 Oil and protein content of the varieties in the final stages of ripening.

VARIETY	DAY	OIL (%)*	TOTAL PROTEIN (%)*	SOLUBLE PROTEIN (%)*
Jet Neuf	31	16.5	10.9	0.69
" "	34	18.5	12.0	0.74
" "	38	22.0	14.6	1.33
" "	41	24.3	16.4	3.50
" "	ripe seed	37.3	25.3	5.00
Lingot	30	20.0	10.8	0.81
"	33	24.1	13.1	1.14
"	37	24.2	13.9	1.25
"	40	26.6	15.9	3.00
"	ripe seed	38.7	23.6	4.30
Jade	30	18.4	12.1	0.83
"	34	22.8	14.3	0.86
"	37	25.0	16.7	1.43
"	42	n.d.	22.0	5.22
"	ripe seed	39.3	26.0	5.08

*Oil and total and soluble protein expressed as % fresh weight

Regarding glucosinolates demolition, expression of myrosinase activity should be a determining factor, especially given its different and significant activity among varieties in early ripening. The trend of myrosinase activity in time supports this idea (Fig.3). In fact,one can easily see that the genotype with the smallest glucosinolate content

shows an enzymatic activity that is significantly higher most of the time during seed ripening (from the 15th to the 35th day), whereas from the 35th day up to complete maturation, cultivar activities tend to mingle. This finding clearly explains the failure of the tests mentioned above aimed at revealing any correlation between myrosinase activity and glucosinolate contents in ripe seeds. This correlation would presumably be detectable if one analyzed immature seeds before the 35th day after the end of anthesis.

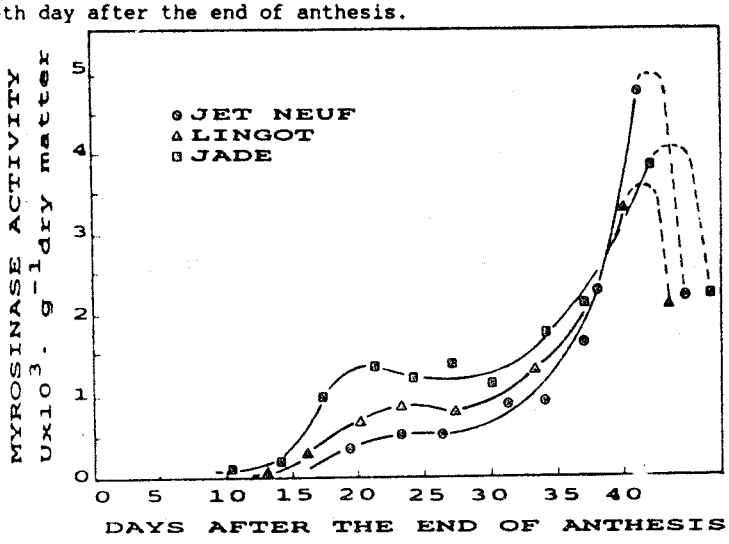

Fig. 3. Myrosinase activity during seed ripening.

If one accepts the hypothesis of myrosinase-regulated glucosinolate content in developing seeds, one must also admit the existence of different isoenzymes, one or more of which should be capable of interacting with glucosinolates in an analogous manner as some factors observed during seed germination by Tookey and Wolff (1970). In this regard we conducted an electrophoretic test of myrosinase extracted and purified by a recently published method (Palmieri et al. 1986) starting with samples from the 30-31st and 33-34th day of ripening for the three genotypes. Fig.4, in addition to revealing the formation of an isoenzyme with low electrophoretic mobility as ripening proceeds, shows a zymogram for Jade that differs constantly in both ripening stages due to the presence of an isoenzyme with higher mobility. We demonstrated the presence of an isoenzyme, or at least a myrosinasic form whose active site conformation is more favorable to ascorbic acid activation, by determining the enzymatic activity at pH 4.5 in the presence of 0.37 mM ascorbic acid. In addition to showing a constantly higher activity for Jade in the control, Table 2 emphasizes the strong activation exerted by ascorbic acid on the myrosinase extracted from this genotype (the activation ratio exceeds 2:1), which is presumably attributable to the characteristic isoenzyme revealed.

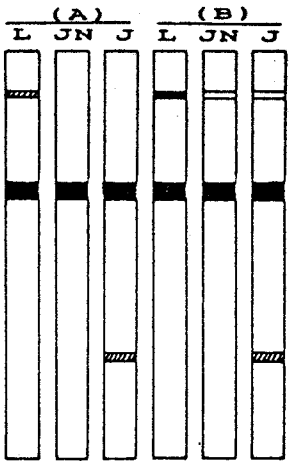

Fig.4 Electrophoretic patterns of myrosinase using PAGE (Davis method) in three varieties at two stages (A and B) of ripening.

L (Lingot)
JN(Jet Neuf)
J (Jade)

TABLE. 2 Effect of ascorbic acid (AA) on the myrosinase activity of three varieties at two ripening stages.

VARIETY	STAGE	DAY	ENZYME ACTIVITY* CONTROL (c)	+AA**(a)	RATIO (c)/(a)
Jet Neuf	A	31	270.2	5,915	21.9
" "	B	34	283.4	5,053	17.8
Lingot	A	30	259.2	4,754	18.3
"	B	33	344.8	4,887	14.2
Jade	A	30	320.7	14,593	45.5
"	B	34	362.4	14,151	39.1

* Enzyme activity expressed as U/g fresh weight
**The concentration of ascorbic acid was 0.375 mM.

Our study provides information on the trends of the main quantitative and qualitative factors during seed maturation and, while preliminary, leads us to believe that: (i) repeated, more detailed studies over the years should permit the identification of agronomic parameters useful for estimating yield; (ii) because it has been demonstrated that there is a constantly higher myrosinase activity for ca. 80% of the detectable ripening period in the genotypes with low and average glucosinolate contents, it should be possible to detect a useful correlation between the parameters in this period ; (iii) the results of electrophoretic and activation tests support the hypothesis of myrosinase-regulated glucosinolate degradation during the ripening process in the 00 varieties, which show a myrosinase with highly specific chemicophysical properties.

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