A SURVEY OF PHENOLIC CHOLINE ESTERS IN BRASSICA AND ALLIED GENERA SEEDS

A. BOUCHEREAU, F. LARHER
Lab. Plant Biology, University of Rennes I, 35042 Rennes France

INTRODUCTION

Among the chemotaxonomical markers which have been used to classify the Crucifereae , sinapine (SIN) was shown to be accumulated in the seeds of most genera (Hegnauer 1964). A few other structurally related compounds were identified in particular cruciferous seeds (Gmelin and Mohrle 1967; Austin and Wolff 1968; Gmelin and Kjaer 1970; Clausen et al. 1982; Pagani 1982; Larsen et al. 1983). These phenolic choline esters (PCE) are of special interest for rapeseed breeders since they are responsible for the desagreeable taste of eggs produced by poultry being fed with rapeseed meal (Clandinin 1960; Hobson-Frohock 1973).Future breeding programms for "low-sinapine" rapeseeds have to rely on basic data about natural variability of distribution patterns of aromatic choline esters in Brassica crops and genetically related species. In support of that, the following investigation was performed on the seeds of various species belonging to : Arabis, Brassica, Cakile, Crambe, Diplotaxis, Eruca, Hesperis, Matthiola, Raphanobrassica, Raphanus and Sinapis genera. Among the Brassica species numerous accessions have been investigated especially for B. napus and related species with the aim to characterize possible genitors for low contents of sinapine and phenolic choline esters. Beside the genetic variabiltiy of PCE accumulation, the fluctuation of the seed sinapine content in 3 rapeseed genotypes was investigated in relation with drought conditions. It is assumed that water availability might have a bearing upon choline esters levels since choline is involved in stress response in a number of plants (Storey and Wyn Jones 1977).

MATERIALS AND METHODS

- **Seeds material** : Most of cultivated types were obtained from I.N.R.A. of Rennes (Le Rheu, France). Wild ecotypes were collected on sand dunes in coastal environments of the west part of France.
- **Cultivation under drought conditions** : The experiment was performed in a greenhouse with 3 rapeseed genotypes : Chine 32, Drakkar and Mali. These genotypes were selected for their different selection geographic area; i.e. China, France and Canada respectively. Plants were grown in pots (d = 25 cm, h = 15 cm) filled with blond peat / brown peat supplemented compost (1 plant per pot). Plans were submitted to water stress at various stages of development and for different treatment durations : B2 stage (stages defined according to C.E.T.I.O.M. 1982) from 36 to 64 DAS (days after sowing); D1-D2 stage from 56 to 80 DAS; F1-G4 stage from 80 to 107 DAS for Chine 32, 84 to 111 DAS for Drakkar and 92 to 119 DAS for Mali.The water shortage was maintained until the shoot water potential reached -20 bars.Sinapine was determined in seeds before experiment and mature seeds collected on control and water stressed plants.
- **Isolation of phenolic compounds and quantitative and qualitative determinations**
Methods and equipment used for isolation, quantitatives and qualitative determinations have been described elsewhere (Bouchereau et al. 1991).

RESULTS AND DISCUSSION

Occurrence of SIN, PCE and related cationic compounds in the seeds of a range of cruciferous plants.

Quite different HPLC chromatograms were obtained for the seeds of the different species under study. A total of 29 peaks of UV light absorbing cationic compounds represented the complete pattern of these components found in seed extracts (figure 1) . Only a few of them were indentified such as 4-hydroxybenzoylcholine, 3,4-dimethoxybenzoylcholine (hesperaline), 3,5-dimethoxy, 4-hydroxycinnamoylcholine (sinapine) and 4-methoxy,3-hydroxycinnamoylcholine (isoferuloyl-choline) . The other peaks characterized by their HPLC chromatographic properties and their UV absorption spectra were attributed either to benzoic acid derivatives or to cinnamic acid derivatives. The relative abundance of the concerned compounds (refering to the peaks area) varied greatly. Sinapine was most abundant and occurred in high concentration in all species examined indicating that SIN is of special interest as a chemotaxonomic marker for the Crucifereae. 4-hydroxybenzoylcholine is also present in most of the collected species. Sinapine-O-β-D-glucopyranoside appears to occur in the seeds of Eruca sativa .Isoferuloylcholine has been identified in a few species i.e. in E. sativa, M. sinuata, R. sativus, D. tenuifolia and D. erucoides . Using HVE, feruloylcholine was shown to occur in M. sinuata, R. sativus niger and many Brassica species. A few peaks arising from unknown compounds were detected only in a restricted number of genera allowing discrimination between or within species. Amphidiploïd species apparently contain the sum of the components detected in their respective diploïd genitors. The absolute amounts of the total PCEs varied in a very wide range with seeds of Arabis alpina exhibiting the lowest value (5 µmols eq. sinapine per g dry seeds) and those of Hesperis matronalis the highest one (55 µmols). The same holds for the sinapine content contributing 9.7 % to the total PCEs in Eruca seeds and 87 % in those of Sinapis alba. The relationship between the SIN and the PCE contents is not so simple since SIN content cannot be deduced from PCE one. With respect to the choline esters pattern among the species studied, we observed divergence between or within species (Figure 2). Thus in the tribe Brassiceae we can easily distinguish the Sinapis group from the Brassica group according to their different PCE content. In a similar way, Arabis, Eruca, Matthiola and Cakile genera are discriminated from more related genera. According to the qualitative pattern of PCEs, Raphanus, Diplotaxis and Hesperis genera seem to be closely related with Brassica group. Interspecific variability is shown to occur in a few genera, i.e. in Sinapis, Diplotaxis and Brassica genera where B. nigra and B. adpressa are clearly separated from the other species. In addition to these genomic relations, analysis of total PCE and SIN contents might provide insight into the impact of environmental conditions on the levels of these compounds in wild and cultivated Brassica and related genera. We observed that seeds of sand dune plants such as Cakile maritima, Crambe maritima, Diplotaxis tenuifolia or Matthiola sinuata exhibited rather low contents of choline esters.

According to these results, PCE content in cruciferous seeds is not only determined by endogenous factors but also regulated by environmental conditions.

Variability of PCE content in the seeds of related Brassica species.

A thorough study of PCE distribution has been performed among the Brassica species (Table1).No cultivar with a very low PCE content has been found in the present study. However, the described variability for PCEs storage capacity is very important since the absolute values fluctuate from 14.9 (B. juncea) to 70.8 µmols per g. dry seeds (B. oleracea). B. oleracea species exhibits the highest values. In addition to differences among species, we also obtained evidence for some intraspecies-specific differences of the PCE pattern. Within the species B. campestris, the subspecies B. c. trilocularis, with yellow seeds, had lower PCE concentrations compared to the other subspecies. Choline esters content in B. napus seeds fluctuate greatly.Glucosinolates and PCE contents are not quantitatively related. However, rapeseed cultivars apparently constitute an intermediate group between cabbages and turnips.Quite a similar situation occurs for B. campestris and B. nigra cultivars. The variability of PCE content in B. juncea species is very remarkable. The presence in this species of numerous types with high PCE contents, as B. nigra, and numerous types with low PCE contents, as B. campestris, support the hypothesis of two geographical forms, i.e. an indian one closely related to B. campestris and an oriental one closely related to B. nigra (Vaughan 1977).Wild forms of B. carinata, an amphidiploïd hybrid between B. oleracea and B. nigra, have never been found (Mizushima and Tsunoda 1967). The cultivated forms are limited to the Ethiopian plateau. This might explain the low variability of PCEs storage ability and the intermediate distribution for Abyssinian mustards.

Water stress effects in sinapine content of rape seeds.

It was found (Figure 3) that in response to a water stress applied at the B2 stage the sinapine content of seeds decreased from 30 to 40 % compared to typical content of seeds from well watered plants. In contrast, when the water deficit was applied at later stages (D1-D2 stages), the sinapine content of seeds was not significantly affected. On the other hand, a water deficit during flowering stages (F1 - G4 stages) induced a significant increase of sinapine in seeds. Thus it can be said that sinapine content of rape seed is under the control of genetic factors the expression of which is found to fluctuate according to water availability. It is well known that water deficit may induce changes in seed composition especially for carbohydrates, oils and proteins (Mailer and Cornish 1987).

CONCLUSION

We have shown that analysis of phenolic choline esters in cruciferous seeds may provide an efficient tool in chemotaxonomy of closely related species in allied genera, especially when genomic relationships cannot be established by classical cytogenetic and morphological methods. This study has also demonstrated the occurrence of previously

identified phenolic choline esters in a few species. We have also pointed out that environmental factors, especially those related to water availability may have a rather important effect upon the accumulation of choline esters in seeds. Concerning the interspecific variability for the capacity to store phenolic choline esters in Brassica species, classified according to U (1935), the levels found in amphidiploïd species ranged between those of the two genitors. We suggest that rapeseed breeders should take into account this opportinity to create "low-sinapine" rapeseeds.

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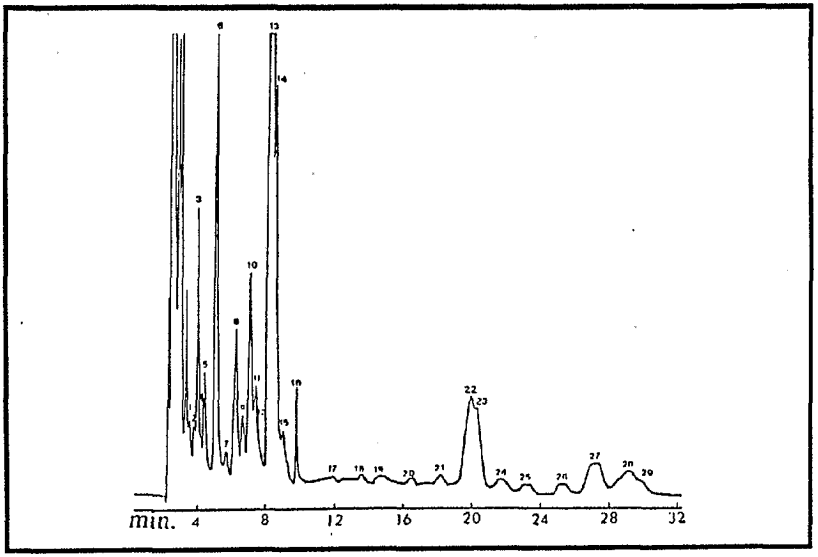

Fig. 1 : Chromatogram of a mixture of all the seeds extracts investigated in this experiment (see Materials and Methods). Support: Spherisorb ODS 2; Column: 120 x 4,6 mm; Mobile phase system as described elsewhere (Bouchereau et al. 1991); Detection: 254 nm.

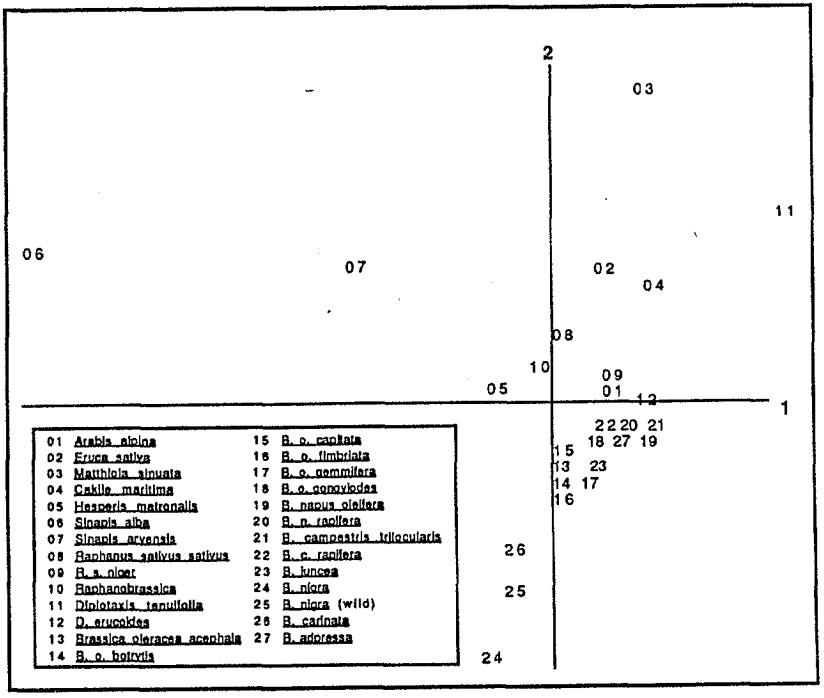

Fig. 2 : Distribution of various members of the Cruciferae on the plane as defined by principal component analysis axis 1-2.

Abscissa : 1 - Axis 1 is related to peaks 4-5-6-7-9-10-22 as defined by HPLC analysis.

Ordinate : 2 - Axis 2 is related to peaks 1-2-14-19-20-21-28 as defined by HPLC analysis.

Table 1 : Mean(m),standard deviation(Sd),maximum level(Cmax) and minimum level(Cmin) of PCE content(µmols.eq.sinapine per g of dry seeds) in seeds of a range of Brassica species.

Plant	n *	m	Sd	Cmax	Cmin
B.oleracea L. var. *botrytis* L.	16	49.6	9.1	70.8	36.2
B.oleracea L. var *acephala* DC.	10	45.1	4.4	51.2	35.6
B.oleracea L. var. *capitata* L.	4	45.8	4.2	50.3	42.6
B.oleracea L. var. *gemmifera* DC.	2	41.6	1.1	42.8	40.5
B.oleracea L. var. *fimbriata* Mill.	1	42.2	/	/	/
B.oleracea L. var. *gongylodes* L.	2	40.7	0.7	41.4	40.0
B.campestris L. var. *trilocularis* (Roxb.)Olsson	9	26.5	2.2	28.0	22.0
B.campestris L. var. *oleifera* Metzg.	2	30.1	1.1	31.2	29.0
B.campestris L. var. *rapifera* Metzg.	7	28.2	4.7	40.7	25.0
B.napus L. var. *oleifera* Metzg.					
Winter types	58	34.6	4.8	47.4	24.9
Spring types	43	36.7	6.0	47.3	27.7
Oil types	87	35.8	5.6	47.4	24.9
Forage types	14	33.7	3.6	39.7	27.7
B.napus L. var. *rapifera* Metzg.	9	35.2	3.9	42.6	28.1
B.juncea L.	28	28.0	7.2	37.1	14.9
B.nigra (L.)Koch.	3	37.7	1.9	40.5	36.0
B.carinata L.	3	40.0	0.7	40.9	39.1

* Number of investigated cultivars

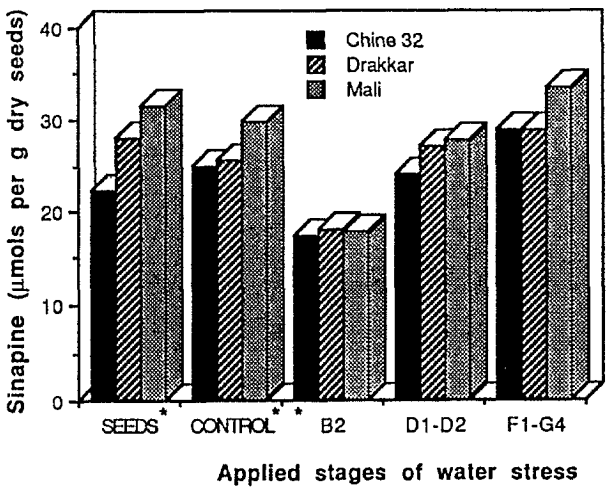

Fig. 3 : Sinapine content in seeds used for the stress experiment (*) and sinapine content in seeds harvested on control plants (**) and treated plants submitted to various water regimes (B2 : water stress applied on B2 stage, D1-D2 : water stress applied on D1-D2 stage, F1-G4 : water stress applied on F1-G4 stage)