Genetic study of very high glucosinolate content in Ethiopian mustard seeds

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Abstract

The Ethiopian mustard (*Brassica carinata* A. Braun) line N2-6215, with very high levels of glucosinolates in the seeds (170 μmol g^{-1} seed), was developed from the line C-101 (125 μmol g^{-1}) following chemical mutagenesis. The objective of this research was to study the inheritance of very high glucosinolate content in seeds of N2-6215. Plants of N2-6215 were reciprocally crossed with plants of two lines with standard (C-101, 127 μmol g^{-1}) and slightly reduced (S2-1241, 115 μmol g^{-1}) glucosinolate content. The F_1, F_2, and BC_1F_1 plant generations were evaluated under the same environment and seeds from individual plants were analysed for total glucosinolate content by near-infrared reflectance spectroscopy. The very high glucosinolate content in N2-6215 seeds was maternally inherited. No cytoplasmic effects were detected. The trait was found to be oligogenic and determined by at least 2-3 genes. The estimates of heritability were 0.31 in the cross with S2-1241 and 0.35 in the cross with C-101. The total glucosinolate content in leaves of the parents, F_1 and F_2 plants from the cross C-101×N2-6215 was measured in order to determine whether the increased glucosinolate levels in the seeds were also expressed at the leaf level. Total glucosinolate content in leaves of the N2-6215 parent was 59 μmol g^{-1}, compared to 52 μmol g^{-1} in leaves of C-101 plants. A moderate but significant correlation (r=0.34) between seed and leaf glucosinolate content was observed in F_2 plants, suggesting that the trait is not seed specific.

Key words: Ethiopian mustard – *Brassica carinata* – seed glucosinolate content – genetic study – leaf glucosinolate content

Introduction

Ethiopian mustard (*Brassica carinata* A. Braun) possesses a number of agronomic advantages over other oilseed *brassica*s in regions with semiarid conditions (Knowles et al., 1981; Fereres et al., 1983). Glucosinolates are secondary plant metabolites with antinutritional effects that adversely affect the nutritional value and organoleptic properties of food and animal feedstuffs (Griffiths et al., 1998). However, glucosinolates and their hydrolysis products possess beneficial properties as well. They show broad biocidal activity which can be used as an alternative to synthetic pesticides for pest and disease control (Kirkegaard et al., 1998; Lazzeri et al., 2004). Furthermore, glucosinolates exert positive influence against biological processes associated with cellular damage and cancer development (Stoewsand, 1995). In general, the genetic control of total glucosinolate content in *Brassica* spp. is complex and controlled by several genes (Hill et al., 2003). The glucosinolate fraction of *B. carinata* seeds is mainly made up of sinigrin (2-propenyl), which accounts for more than 95% of the total glucosinolates in the seeds (Getinet et al., 1997). Although the genetic control of sinigrin synthesis in *B. carinata* has not been studied, the trait has been found to be controlled by three genes both in resynthesized *B. napus* forms (Gland, 1985) and in *B. juncea* (Mahmood et al., 2003).

The Ethiopian mustard line N2-6215, characterized by very high levels of glucosinolates in the seeds (170 μmol g^{-1} seed), was developed from the line C-101 (127 μmol g^{-1}) following chemical mutagenesis. The objective of the present research was to study the inheritance of very high glucosinolate content in N2-6215 seeds and to evaluate the expression of the trait at the leaf level.

Material and Methods

Plant Material: C-101 is a standard Ethiopian mustard line with an average glucosinolate content of around 127 μmol g^{-1}. The lines S2-1241, with slightly reduced glucosinolate content (115 μmol g^{-1}) and N2-6215, with very high glucosinolate content (170 μmol g^{-1}), were developed from C-101 following chemical mutagenesis and pedigree selection (Velasco et al., 1996; 1999). Seeds of the three parents were sown in December 2002 and the plants were grown in pots in a screenhouse. Plants of N2-6215 were reciprocally crossed with plants of S2-1241 and C-101 in the spring of 2003. Microperforated plastic bags were used to prevent uncontrolled pollination. Crossing was done by emasculating immature flower buds of the female parent followed by immediate pollination of their stigmas with fresh pollen from open flowers of the male parent. F_1 plants were grown in the screenhouse in the spring of 2004 together with parent plants. They were self-pollinated to obtain F_2 seed and also backcrossed to both parents. The F_1, F_2 and BC_1F_1 plant generations were grown in the field together with plants of the parents in the spring of 2005. All the plants were bagged before flowering. Young leaves from the parents and the F_1 and F_2 generations of the cross C-101×N2-6215 were collected before flowering for the analysis of total glucosinolate content. Seeds from individual plants were harvested at maturity.

Seed glucosinolate analyses: Total glucosinolate content was measured on intact seeds by near-infrared reflectance

spectroscopy (NIRS) with the pd-glucosinolate complex method (Thies, 1982) used as reference method for NIRS calibration (Velasco et al., 1999).

Leaf glucosinolate analyses**:** Young leaves, frozen at -80 ºC immediately after collection, were freeze dried and ground in a lab mill. The total glucosinolate content in the leaves was measured using the pd-glucosinolate complex method (Thies, 1982).

Statistical analyses: Maternal and cytoplasmic effects were studied in F_1 and F_2 seeds, respectively. The minimum number of genes k controlling total glucosinolate content was estimated following Wright (1968): $k = (P_1-P_2)^2/8(S^2F_2-V_E)$, where P_1 and P_2 are the mean values of the two parents, S^2F_2 is the variance of the F_2 plants and V_E the environmental variance. V_E was estimated by pooling the variances within the genetically uniform generations P_1, P_2 and F_1. The heritability of total glucosinolate content (h^2) was calculated as the ratio of the additive genetic variance to the total phenotypic variance (Sánchez-Monge and Jouve, 1989).

Results and Discussion

The Ethiopian mustard lines S2-1241, C-101 and N2-6215 showed average glucosinolate contents of 94, 113 and 183 μmol g^{-1}, respectively. F_1 seeds from the cross S2-1241×N2-6215 averaged 124 μmol g^{-1}, whereas those from the reciprocal cross averaged 214 μmol g^{-1}. F_1 seeds from the cross C-101×N2-6215 averaged 170 μmol g^{-1} whereas those from the reciprocal cross averaged 207 μmol g^{-1}. The results indicated a strong maternal effect on total glucosinolate content.

At the F_1 plant level (F_2 seeds averaged), no significant differences between reciprocal crosses were observed (Table 1), indicating absence of cytoplasmic effects on this trait. The existence of cytoplasmic effects on total glucosinolate content in *Brassica* species is a matter for controversy, as they have been identified in some studies (Kondra and Stefansson, 1970; Love et al., 1990) but not in others (Rücker and Röbbelen, 1994).

Table 1. Glucosinolate content (μmol g^{-1}) in the *Brassica carinata* lines S2-1241, C-101, N2-6215, and F_1 plants from crosses between them.

Generations	Glucosinolate content (μmol g^{-1} seed)	
	Screenhouse 2004	Field 2005
Parents		
S2-1241	49 ± 7	115 ± 9
C-101	87 ± 13	127 ± 14
N2-6215	146 ± 23	170 ± 10
F_1 plants		
S2-1241×N2-6215	96 ± 15[a]*	158 ± 5[b]
N2-6215×S2-1241	98 ± 22[a]	153 ± 10[b]
C-101×N2-6215	90 ± 20[c]	157 ± 8[d]
N2-6215×C-101	102 ± 18[c]	157 ± 10[d]

*Within the same cross and environment, averages followed by the same letter are not statistically different ($P \leqslant 0.01$).

F_2 plants exhibited a great variation for glucosinolate content in both crosses, from 106 to 184 μmol g^{-1} in the cross S2-1241×N2-6215, and from 108 to 180 μmol g^{-1} in the cross C-101×N2-6215 (Fig. 1). Glucosinolate content in both F_2 populations showed continuous distributions, which was also the case for the BC_1F_1 populations (Fig. 1). Estimates of heritability resulted in values of 0.31 in the cross S2-1241×N2-6215 and 0.35 in the cross C-101×N2-6215, indicating an important environmental effect on total glucosinolate content in Ethiopian mustard seeds. Previous studies in *B. napus* have reported higher values of heritability (Rücker and Röbbelen, 1994), although they included parents with a low glucosinolate content (⩽10 μmol g^{-1}).

The estimates of the number of genes controlling differences in glucosinolate content were obtained from the frequency distributions of parents, F_2 and BC (Fig. 1). The results indicated that at least 2-3 genes must be responsible for the very high glucosinolate (sinigrin) content in N2-6215. There are no previous studies on the inheritance of very high glucosinolate content in *Brassica* species. Genetic studies on total glucosinolate content including parents with low and high glucosinolate content have reported the presence of 2-3 segregating genes (Siebel and Pauls, 1989; Love et al., 1990) or even a greater number of genes (Kondra and Stefansson, 1970; Busch and Röbbelen, 1981; Toroser et al., 1995; Mahmood et al., 2003; Rücker and Röbbelen, 1994). In relation to the inheritance of sinigrin content, the trait has been found to be controlled by three genes both in resynthesized *B. napus* forms (Gland, 1985) and in *B. juncea* (Mahmood, et al. 2003).

Leaves of N2-6215 also expressed a higher glucosinolate content (59 ± 4 μmol g^{-1}) than leaves of C-101 (52 ± 10 μmol g^{-1}), whereas leaves of F_1 plants had an intermediate glucosinolate content (55 ± 4 μmol g^{-1}). Glucosinolate content in the leaves of F_2 plants ranged from 20 to 63 μmol g^{-1}. A moderate but significant correlation ($r=0.34$, $p\leqslant0.01$) between seed and leaf glucosinolate content was observed in F_2 plants (Fig. 2). This correlation is lower than that reported in studies in *B. napus* ($r>0.85$) (Jürges, 1982; Schilling and Friedt, 1991).

There are interesting applications for *Brassica* germplasm with very high glucosinolate content, for example for use as green manure with natural biocidal activity (Lazzeri et al., 2004). The Ethiopian mustard line N2-6215, with a very high seed

glucosinolate content that is also expressed at the leaf level, has a great potential for such applications.

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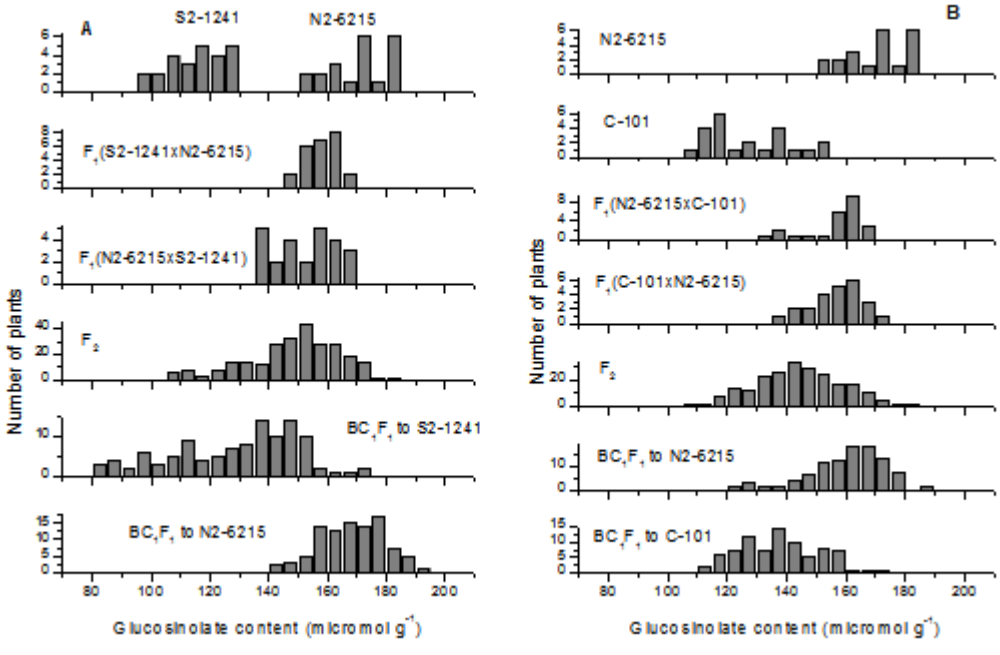

Fig. 1. Frequency distributions of glucosinolate content (μmol g^{-1} seed) in individuals plants of S2-1241, N2-6215, and F_1, F_2, and BC_1F_1 populations from crosses S2-1241×N2-6215 (A) and N2-6215×C-101 (B).

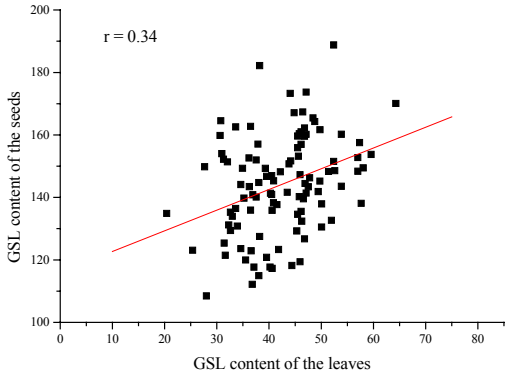

Fig. 2. Seed and leaf total glucosinolate (GSL) content (μmol g^{-1}) in F_2 plants from cross N2-6215×C-101.