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Seed longevity in oilseed rape (*Brassica napus* L.) – genetic variation and QTL mapping

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Abstract

Although oilseed rape has become one of the most important oil crops in Europe, little is known regarding the viability of its seed under conditions of long-term storage. We report here an examination of oilseed rape seed longevity performed on a set of 42 accessions housed at the German *ex situ* genebank at IPK, Gatersleben. A comparison of germination between the accessions stored for 26 years showed that viability was in part genetically determined, since it ranged between 42 and 98%. An attempt was made to define the genetic basis of viability by subjecting a mapping population of doubled haploids to three artificial ageing treatments. Quantitative trait loci (QTL) were detected on six chromosomes: N6, N7, N8, N15, N16 and N18. The chromosomal locations of these QTL were compared with their syntenic regions in *Arabidopsis thaliana* in order to explore what genes might underlie genetic variation for longevity.

Keywords: *Brassica napus* L; *ex situ* genebank; quantitative trait loci; seed longevity; synteny

Introduction

Originally developed as a source of oil for energy purposes, oilseed rape (*Brassica napus* L.) has now risen to become one of the most important field crops in Europe, Canada, China and Australia. Its oil is now used in many applications, including biodiesel, culinary oil and livestock feed. Due to its remarkable increase in production, canola has become the focus of much breeding and molecular genetics in recent years (Friedt and Snowdon, 2010).

Until recently, the longevity of *B. napus* seed has been considered to be of little importance. However, as particularly the spring-type canola crop becomes transgenic-based, some concern has been expressed about the growing dominance of transgenic material in the soil seed bank, while at the same time, there is a continuing loss of non-transgenic materials stored in genebanks, as a result of ageing. Seeds from *Brassica* spp. typically lose ~50% of their viability with 7.3 years of storage at 20°C and 50% relative humidity (RH) (Nagel and Börner, 2010), and within 23 years under standard low temperature (−18°C) storage conditions (Walters *et al.*, 2005a). Evidence that seed viability in some crop species is in part genetically determined (Nagel *et al.*, 2010) has prompted the present study of intra-specific variation in *B. napus* seed longevity.

Materials and methods

We tested the seed viability of 42 accessions of *B. napus* ssp. *napus* var. *napus* f. *biennis*, which had been

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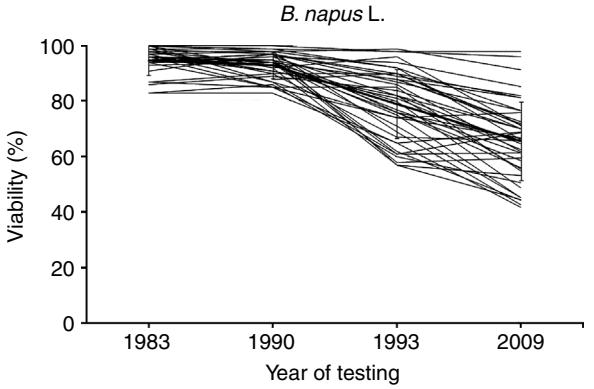


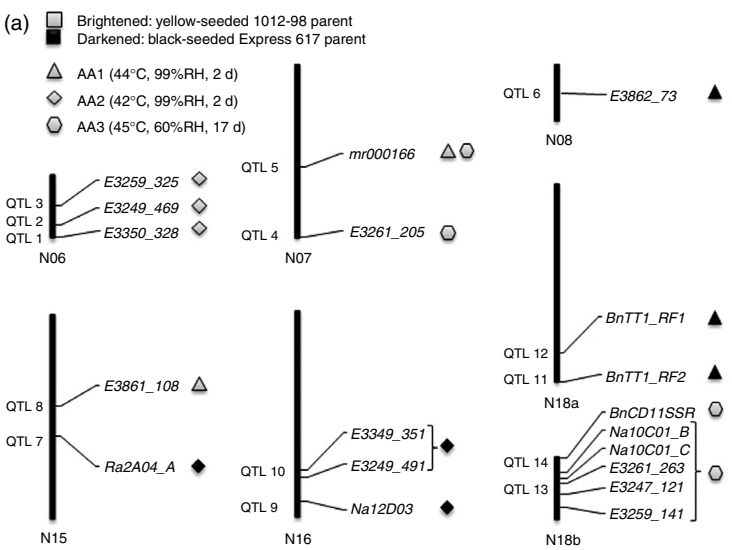
Fig. 1. Mean viability of *B. napus* genebank accessions over different test years. The bold line indicates mean viability, and standard deviations over the years are shown.

multiplied in 1983 and have been maintained in the interim at 7 ± 3°C and 6 ± 2% seed moisture content. Three replicates of 50 seeds/accession were placed on moistened filter paper and germination was monitored after 28 d. Their current viability was compared with historic data collected in 1983, 1990 and 1993 by using arithmetic means and standard deviations.

A set of artificial ageing protocols was applied to a population of 153 doubled haploid lines of the winter oilseed rape YE2-DH mapping population (Badani *et al.*, 2006) to explore the genetic basis of seed longevity. The protocols were: AA1, following Hampton and TeKrony (1995), in which two replicates of 50 seeds each were sealed in glass jars containing 200 ml deionised water to raise the RH above 99%. After holding the jars for 48 h at 42°C, a germination test was conducted as above, with a final count after 7 d. AA2: this was identical to AA1, except that the temperature was 44°C. AA3: following Hay *et al.* (2008), in which two replicates of 100 seeds/line were placed inside a sealable box containing a 8.7 M LiCl solution which ensured that the RH at 20°C stabilized at 47% within 14 d. Artificial ageing was then initiated by changing the solution (7.1 M LiCl), and maintaining the temperature at 45°C (to give an RH of 60%) for 17 d. This was followed by the same germination test as above. A measure of relative viability for each method was calculated by dividing the viability of the aged seeds by their initial viability. The data were subjected to a quantitative trait loci (QTL) analysis, using QGENE software (Nelson, 1997) and a genetic map of, in total, 161 markers comprising amplified fragment length polymorphism and simple-sequence repeat markers (Snowdon *et al.*, unpublished data). A permutation test was used to set the appropriate logarithm of odds (LOD) ratio threshold for each treatment.

Results and discussion

The decay in viability of the genebank accessions over 26 years of storage is shown in Fig. 1. The mean proportion of viable seeds fell from 94.7% in 1983 to 92.9, 79.1 and 65.7% after 7, 10 and 26 years of storage, respectively. The associated standard deviations were 5.4% (1983) 4.6% (1990), 12.5% (1993) and 14.2% (2009). Despite having been grown simultaneously and subjected to the same post-harvest and storage conditions, the accessions nevertheless displayed variation with respect to seed viability, as also occurs in barley, wheat, sorghum, rye and



(b)

QTL	Treatment	Marker	LOD score	R^2 value
1	AA2	*E3350_328*	1.53**	0.049
2	AA2	*E3249_469*	1.25*	0.040
3	AA2	*E3259_325*	1.19*	0.038
4	AA3	*E3261_205*	1.90*	0.056
5	AA1	*mr000166*	0.87*	0.028
5	AA3	*mr000166*	1.24*	0.039
6	AA1	*E3862_73*	0.88*	0.027
7	AA2	*Ra2A04_A*	1.30*	0.041
8	AA1	*E3861_108*	0.98*	0.030
9	AA2	*Na12D03*	1.13*	0.037
10	AA2	*E3349_351*	1.29*	0.042
10	AA2	*E3249_491*	1.24*	0.040
11	AA1	*BnTT1_RF2*	1.09*	0.062
12	AA1	*BnTT1_RF1*	1.05*	0.061
13	AA3	*Na10C01_C*	2.24**	0.067
13	AA3	*E3261_263*	2.02*	0.060
13	AA3	*Na10C01_B*	1.85*	0.055
13	AA3	*E3247_121*	1.34*	0.040
13	AA3	*E3259_141*	1.27*	0.038
14	AA3	*BnCD11SSR*	2.68***	0.081

*,**,*** Significant at $P = 0.05$, $P = 0.01$ and $P = 0.001$, respectively.

Fig. 2. QTL interval mapping in the *B. napus* YE2-DH population detected a range of loci with effects on seed longevity after artificial ageing. The choice of artificial ageing protocol (AA1, AA2 and AA3) applied was found to be important. The 14 QTL map to seven different chromosomes (a). Individual QTL effects are tabulated (b).

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linseed (Nagel *et al.*, 2009, 2010). We therefore concluded that there is a genotypic component involved in the determination of seed viability.

When the doubled haploid lines from the YE2-DH population were tested using the three artificial ageing methods, 13 significant QTL affecting seed longevity were identified (Fig. 2). Most of the QTL were method-specific. AA1 produced five QTL, mapping to chromosomes N7, N8, N15 and N18a, while AA2 generated six QTL on N6, N15 and N16. By using AA3, four QTL on chromosomes N7 and N18b were found with in particular highest explained phenotypic variation in QTL 14: $R^2 = 0.081$. The only QTL common to AA1 and AA3 was QTL5, on chromosome N7.

The reliability of artificial ageing as a surrogate for long-term storage has been repeatedly discussed in the literature (Delouche and Baskin, 1973; Priestley and Leopold, 1979; McDonald, 1999; Freitas *et al.*, 2006), leading to a number of mutually inconsistent conclusions. Rajjou *et al.* (2008) suggested that a controlled deterioration test protocol, as described by Tesnier *et al.* (2002), mimics many of the molecular and biochemical events experienced during seed ageing. On the other hand, in this study, we showed that even a modest increase in ageing temperature (from 42 to 44°C) can have a major effect on the expression of relevant genes. The QTL identified in AA1 and AA2 mapped to distinct regions from those detected following AA3, which compared with AA1 and AA2 operates on lower seed moisture contents in a dry state, according to Walters *et al.* (2005b).

The *Brassica* consensus map of Parkin *et al.* (2005) was used to estimate potential locations of orthologous loci in the *Arabidopsis thaliana* genome with respect to the *B. napus* QTL. Potential links with *B. napus* QTL were found for the upper part of the *A. thaliana* chromosomes AtC1 and AtC3, and near the lower ends of AtC1 and AtC5. The genetic basis of seed longevity in *A. thaliana* has been ascribed to genes mapping in the upper parts of chromosomes AtC1 and AtC3 (Bentsink *et al.*, 2000; Clerkx *et al.*, 2004b). Other QTL related to germination in the presence of salinity or heat stress, as well as some controlling the rate of germination, are also known in the region of AtC1, which suggests the possibility that tolerance to stress represents an aspect of seed longevity (Clerkx *et al.*, 2004b). Nevertheless, many of the genes involved in the stress response are also participants in the oxidative stress response. An example of this connection has been provided by Thorlby *et al.* (1999), who detected the expression of genes in the region of AtC1 related to tolerance of oxidative stress, as well as of some involved in abscisic acid (ABA) biosynthesis and perception. *A. thaliana* mutants which have lost sensitivity to ABA, or are compromised in its synthesis, tend to show poor seed longevity (Clerkx *et al.*, 2004a). This demonstrates that ABA plays a role in maintaining seed viability during storage. Certain chemical and/or physical properties of the seed-coat also affect germination rate after storage, since the seed of both structural and pigmentation mutants tends to deteriorate faster than that of their wild-type progenitor (Debeaujon *et al.*, 2000; Clerkx *et al.*, 2004a).

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