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Full Length Research Paper

Phylogenetic relationships within and among *Brassica* species from RAPD loci associated with blackleg resistance

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The genus *Brassica* comprises economically important oilseed and vegetable crops. Their susceptibility to fungal diseases such as blackleg causes yield loss. In this study, thirty accessions from USDA germplasm collection representing two diploid *Brassica* species (*Brassica rapa* and *Brassica oleracea* var. *virids*) and fifteen tetraploid cultivars (*Brassica napus*) from the national winter canola variety trials (NWCVT) were evaluated using 13 sets of random amplified polymorphic DNA (RAPD) associated with blackleg resistance in *Brassica nigra*. 126 highly polymorphic bands with an average of 10 per primer were detected. A UPGMA dendrogram showed *B. rapa* as highly diverse and was supported from three different basal branches, while *B. napus* accessions were generally monophyletic. Similarly, all of *B. oleraceae* accessions were supported from the same basal node. Generally, the three species were reciprocally paraphyletic, suggesting that the RAPD markers showed both functional relationships as well as homology, possibly due to selection at the RAPD loci associated with blackleg resistance. Consequently, two potentially susceptible *B. napus* accessions were identified. The high polymorphic information content (PIC) and number of phylogenetically informative bands established RAPD as a useful tool for phylogenetic reconstruction, quantification of genetic diversity for conservation, cultivar classification and molecular breeding in *Brassica*.

Key words: Blackleg, phylogeny, polyploid, homolog, RAPD, functional relationship.

INTRODUCTION

Oilseed rape (*Brassica napus*) is an important source of edible oil and dominates the world's cultivated *Brassica* (Banga 1993; Mailer et al., 1994). The genus *Brassica* includes economically important oilseed and vegetable crops such as cabbage, cauliflower, kale, broccoli, turnip rape, oilseed mustards and oilseed rape. Diploid species in this genus are cytogenetically classified as A, B, and C genomes, whereas amphidiploids contain AB, BC or AC genomes (U, 1935). Diploid species include *Brassica rapa* (A; n = 10), *Brassica nigra* (B; n = 8) and *Brassica oleracea* (C; n = 9), whereas amphidiploids comprise *Brassica juncea* (A+B; n = 18), *Brassica carinata* (B+C; n = 17) and Oilseed rape, *B. napus* (A+C; n = 19). The gene pool of elite oilseed rape breeding material has been considerably reduced by emphasis on specific quality traits (Snowdon and Friedt, 2004). This narrow genetic base has increased their vulnerability to diseases. Many important fungal pathogens reside in the soil and infect roots, leaves, stems and fruits of crops, often causing significant yield losses. Fungal diseases such as white rust, blackspot, clubroot, sclerotinia, and blackleg (Ananga et al., 2006) and bacterial leaf spot, black rot, and soft rot (Rimmer and Buchwaldt, 1995), as well as insect pests such as aphids, Japanese-beetle and cabbage seedpod weevil (Salisbury et al., 1995) pose a

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major limitation to the cultivation of this important crop.

Despite being generally susceptible, a growing body of evidence show that some oilseed rape cultivars are resistant to blackleg disease (Ansan-Melayah et al., 1995; Ansan-Melayah et al., 1997a; Ananga et al., 2006). Specifically, species that contain the B genome (*B. juncea*, *B. nigra* and *B. carinata*) can display total resistance to blackleg disease throughout the life of the plant (Roy, 1984; Ananga et al., 2006). Resistant oilseed rape lines have also been obtained through interspecific crosses between *B. napus* and *B. nigra* (Rimmer et al., 1995; Chevre et al., 1997; Brun et al., 2001). Hence the use of resistant cultivars has been advocated as the most effective way of limiting yield losses due to blackleg disease (Rimmer et al., 1995).

Identification of resistant cultivars can involve either field trials (disease challenge and plant response data), or predictions based on genetic relationships. Field trials suffer practical drawbacks such as longer time to obtain results, are resource intensive, and the results may be unreliable when the trait is under a strong environmental influence, unlike molecular genetic analyses. Therefore, analysis of the genetic variation and relatedness in the *Brassica* germplasm is needed for genetic resource conservation and plant breeding programs. Resistance to plant pathogens has been shown to be under genetic control (Brun et al., 2001), hence the genetic relationships among taxa can be used to predict susceptibility or resistance (Ochieng et al., 2007a). Understanding the interspecific and intraspecific relationships among cultivated species of *Brassica* will inform better parental selection and to widen the genetic basis of resistance to blackleg through greater variations and the development of new genotypes in oilseed rape cultivars.

Studies on a broader phylogenetic relationships among diploid species including *B. nigra, B. rapa,* and *B. oleracea*, showed that the B genome (*B. nigra*; B) was supported from a distinct clade separate from the other two (*B. rapa*; A, and *B. oleracea*; C) (Inaba and Nishio, 2002). There is no reported phylogenetic placement of cultivated *B. napus* among these species, however, the study of Inaba and Nishio (2002) would appear to suggest that *B. napus*, a tetraploid containing AC genome, is allied to the *rapa-oleracea* clade. The present study assessed the phylogenetic relationships within and among cultivated *B. napus, B. rapa*, and *B. oleracea* using RAPD markers associated with blackleg resistance in *B. nigra* (Ananga et al., 2006). Phylogenetic placement of oilseed rape cultivars from the NWCVT in relation to resistant or susceptible cultivars (Ananga et al., 2006) can help predict their potential response to blackleg. Because these RAPD markers are associated with resistance to a plant pathogen, the resultant phylogenies are predicted to reflect functional genetic variation and affinities. Since the diploid species *B. rapa* and *B. oleracea* each represent a parental genome of the amphidiploid species, the placement of *B. napus*, *B. rapa*, and *B. oleraceae* cultivars within the *rapa-oleraceae* clade may identify unique variants needed in breeding programmes, expansion of the range of useful variation, and for future improvement of heterotic potential.

MATERIALS AND METHODS

Sample collection, DNA extraction and PCR amplification

Forty-five plant materials were used in this analysis. The samples comprised 15 accessions each of *B. rapa* and *B. oleracea* var. *virids* from the USDA germplasm collections (Fort Collins, Colorado), and 15 cultivars of *B. napus* from the National Winter Canola Variety Trials (NWCVT, Kansas) (Table 1). More detailed information on the accessions is available at the USDA-ARS Germplasm Resources Information Network available online at (http://www.ars-grin.gov/npgs). Genomic DNA was extracted from leaves of five-week old seedlings according to Edwards et al. (1991). Thirteen polymorphic RAPD primers: OPB01, OPB05, OPC08, OPE03, OPE11, OPE12, OPE14, OPE16, OPF02, OPF10, OPG02, OPT01 and OPI01 mapped to Chromosome 4 of B. nigra (Chevre et al., 1997), were selected for this study. The selected markers were obtained from MWG-Biotech (High Point, NC) for this study. The identity and sequence information for each marker are given in Table 2. PCR reactions were carried out in 25 µL volumes comprising the following: 0.4 mM of each dNTP (Promega), 1.5 mM MgCl2, 1.25 units of gold Taq DNA polymerase (Promega, Madison, WI), 0.4 pmol of each primer, 1x PCR buffer and 25 ng DNA template. The amplifications were performed on a Peltier Thermal Cycler (PTC-200; MJ Research). Amplifications comprised 45 cycles each having the following repeated sequence: Denaturation at 94ºC for 1 minute, annealing at between 32 and 34ºC for 1 min depending on the marker (Table 2), extension at 72ºC for 2 min. A final extension at 72ºC for 7min was applied to all reactions. Amplified PCR products, mixed with a loading dye (0.25% bromophenol blue, 0.25% xylene cynol FF, 15% Ficoll) to increase its density and aid visualization, were loaded onto 1% agarose. A Bench-top 1 Kb DNA ladder (Promega, Madison, WI-Catalog no. G7541) was loaded alongside the PCR products for size calling. The fragments were separated under electric current for 4 h in a standard horizontal electrophoresis unit, followed by a 15 min ethidium bromide staining. Gels were de-stained and visualized under UV light. Gel images were captured using AlphaImager v5.5 2000 (Alpha Innotech Corporation, San Leandro, CA).

Statistical data analyses

Amplified DNA fragments were evaluated for size in base pairs using AlphaEaseFC version 4.0.1 (Alpha Innotech Corporation, San Leandro CA). Polymorphic information content (PIC) values were calculated for each primer as demonstrated by Botstein et al., 1980 and also by using the online resource, Polymorphism Information Content Calculator (http://www.agri.huji.ac.il/~weller/Hayim/parent/PIC.htm). PIC compares the polymorphism levels across markers (Hongtrakul et al., 1997; Garcia et al., 2004) and is used to determine the usefulness of markers for specific studies. Polymorphic bands were scored as binary data (present = 1; absent = 0) across the genotypes to generate a binary data matrix. The matrix was then used to generate a Genetic similarity (GS) matrix based on Jaccard's coefficient of similarity (Jaccard, 1908). In brief, GS (ij) = 2a/(2a + b + c), where GS (ij) is the measure of genetic similarity between individuals i and j, a is the number of polymorphic bands that are shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i. The matrix of

Table 1. *Brassica* accessions, species, genomic group and source/origin used in the study.

No.	Selected Brassica	Species	Genome	Origin
1	NSL 6115	*Brassica rapa*	AA	USA, New York
2	NSL 6119	*Brassica rapa*	AA	USA, New York
3	NSL 167291	*Brassica rapa*	AA	USA, New York
4	NSL 6105	*Brassica rapa*	AA	USA, Virginia
5	NSL 6106	*Brassica rapa*	AA	USA, Virginia
6	NSL 6109	*Brassica rapa*	AA	USA, Virginia
7	NSL 6110	*Brassica rapa*	AA	USA, Virginia
8	NSL 6111	*Brassica rapa*	AA	USA, Virginia
9	NSL 6112	*Brassica rapa*	AA	USA, Virginia
10	NSL 6114	*Brassica rapa*	AA	USA, Virginia
11	NSL 6120	*Brassica rapa*	AA	USA, Virginia
12	NSL 6121	*Brassica rapa*	AA	USA, Virginia
13	NSL 6122	*Brassica rapa*	AA	USA, Virginia
14	NSL 6125	*Brassica rapa*	AA	USA, Virginia
15	NSL 6126	*Brassica rapa*	AA	USA, Virginia
16	Abilene	*Brassica napus*	AACC	NWCVT, Kansas
17	Arctic	*Brassica napus*	AACC	NWCVT, Kansas
18	Baldur	*Brassica napus*	AACC	NWCVT, Kansas
19	Banjo	*Brassica napus*	AACC	NWCVT, Kansas
20	Ceres	*Brassica napus*	AACC	NWCVT, Kansas
21	Jetton	*Brassica napus*	AACC	NWCVT, Kansas
22	Kronos	*Brassica napus*	AACC	NWCVT, Kansas
23	Maestro	*Brassica napus*	AACC	NWCVT, Kansas
24	Plainsman	*Brassica napus*	AACC	NWCVT, Kansas
25	Rasmus	*Brassica napus*	AACC	NWCVT, Kansas
26	Talent	*Brassica napus*	AACC	NWCVT, Kansas
27	Titan	*Brassica napus*	AACC	NWCVT, Kansas
28	Viking	*Brassica napus*	AACC	NWCVT, Kansas
29	Wotan	*Brassica napus*	AACC	NWCVT, Kansas
30	Wichita	*Brassica napus*	AACC	NWCVT, Kansas
31	NSL 6144	*Brassica oleracea*	CC	USA, Virginia
32	NSL 6145	*Brassica oleracea*	CC	USA, Virginia
33	NSL 6146	*Brassica oleracea*	CC	USA, Virginia
34	NSL 6147	*Brassica oleracea*	CC	USA, Virginia
35	NSL 6149	*Brassica oleracea*	CC	USA, Virginia
36	NSL 6151	*Brassica oleracea*	CC	USA, Virginia
37	NSL 6153	*Brassica oleracea*	CC	USA, Virginia
38	NSL 67978	*Brassica oleracea*	CC	USA, Virginia
39	NSL 67979	*Brassica oleracea*	CC	USA, Virginia
40	NSL 6150	*Brassica oleracea*	CC	USA, Illinois
41	NSL 6143	*Brassica oleracea*	CC	USA, Missouri
42	NSL 6154	*Brassica oleracea*	CC	USA, Missouri
43	NSL 6148	*Brassica oleracea*	CC	USA, Pennsylvania
44	NSL 80301	*Brassica oleracea*	CC	USA, Pennsylvania
45	NSL 80302	*Brassica oleracea*	CC	USA, Pennsylvania

Table 2. Size range, polymorphic information content (PIC) and number of fragments (NOF) detected at the RAPD markers.

No.	RAPD Name	Size range (bp)	PIC across taxa	Number of fragments			
				B. oleracea	*B. rapa*	*B. napus*	Across taxa
1	OPB01	125-3500	0.97	8	10	10	9
2	OPB05	300-3400	0.83	7	9	13	10
3	OPC08	140-1570	0.74	8	6	9	8
4	OPE03	600-4000	0.94	6	6	7	6
5	OPE11	235-2250	0.92	8	13	12	11
6	OPE12	690-2000	0.88	8	11	7	9
7	OPE14	390-2770	0.79	8	12	7	9
8	OPE16	600-2500	0.86	13	12	13	13
9	OPF02	160-2600	0.84	6	12	7	8
10	OPF10	400-3500	0.78	13	11	13	12
11	OPG02	320-3300	0.77	8	10	10	10
12	OPT01	210-2700	0.84	11	9	8	9
13	OPI01	280-2500	0.92	11	12	13	12
	Total			122	124	123	126
	Mean		0.85	9	10	10	10

PIC = Polymorphic information content; bp = base pairs.

Genetic similarity was used to construct a dendrogram using the unweighted pair group method with arithmetic averages (UPGMA) algorithm.

RESULTS AND DISCUSSION

RAPD markers were highly polymorphic

All the RAPD markers were polymorphic with a total of 126 polymorphic fragments detected. Electrophoretic fragments ranged in size from 125 to 4000 bp. This range is consistent with those in a previous study of genetic diversity in *B. oleracea* and their wild relatives (Lazaro and Aguinagalde, 1998). The number of polymorphic fragments detected varied across loci; with the lowest being six (OPE03), while the highest was 13 (OPE16). The average number of polymorphic bands per primer was 10 (Table 2). Polymorphic information content (PIC) values ranged from 0.78 (OPF10) to 0.97 (OPB01) with an average PIC of 0.85 (Table 2). *B. rapa* showed considerable diversity whereas *B. oleraceae* had the lowest diversity. The very narrow diversity among *B. oleraceae* accessions may have resulted from artificial selection and breeding practices, consistent with earlier reports that the gene pool of elite oilseed rape breeding material has been considerably reduced by emphasis on specific quality traits (Snowdon and Friedt, 2004). The efficiency of RAPD markers in revealing a great number of bands per reaction (Mailer et al., 1994), the lower costs (no need to characterize new genomes) and the simplistic instrumentation required, make these markers suitable for phylogenetic reconstructions, cultivar or variety identification in molecular breeding.

Basal nodes were generally homologous

The term homology implies different things to different disciplines. However, variation in the use of this term is notable within evolutionary biology literature. Homology is often used to refer to sequences, morphs, characters that show similarity. However, in its strict sense, homologs are not only similar, but they necessarily show evidence of descent from a common ancestor. Thus homologs are characters that are similar enough to have come from a common ancestry. In this context, homology would be concluded for a clade (monophyletic group). Nearly all the major nodes supported members of the same taxonomic classification. The most notable example was the node supporting the cultivated *B. napus* species (AC genome), where 13 out of 15 accessions were supported from the same basal node (Figure 1).

Brassica species were paraphyletic

Cladistics defines a paraphyletic group as a group whose members are descended from a common ancestor, but which does not include all of the known or considered descendants of that common ancestor (Brummit, 2002). In this study, a phylogenetic definition is implied: “paraphyletic” means a “clade” that includes within it, a non-descendant of their most recent common ancestor; whereas a monophyletic group is a group consisting of members descended from a single most recent common ancestor, (Ochieng et al., 2007a). The dendrogram (Figure 1) shows all the three *Brassica* species to be reciprocally paraphyletic. For example, Accessions

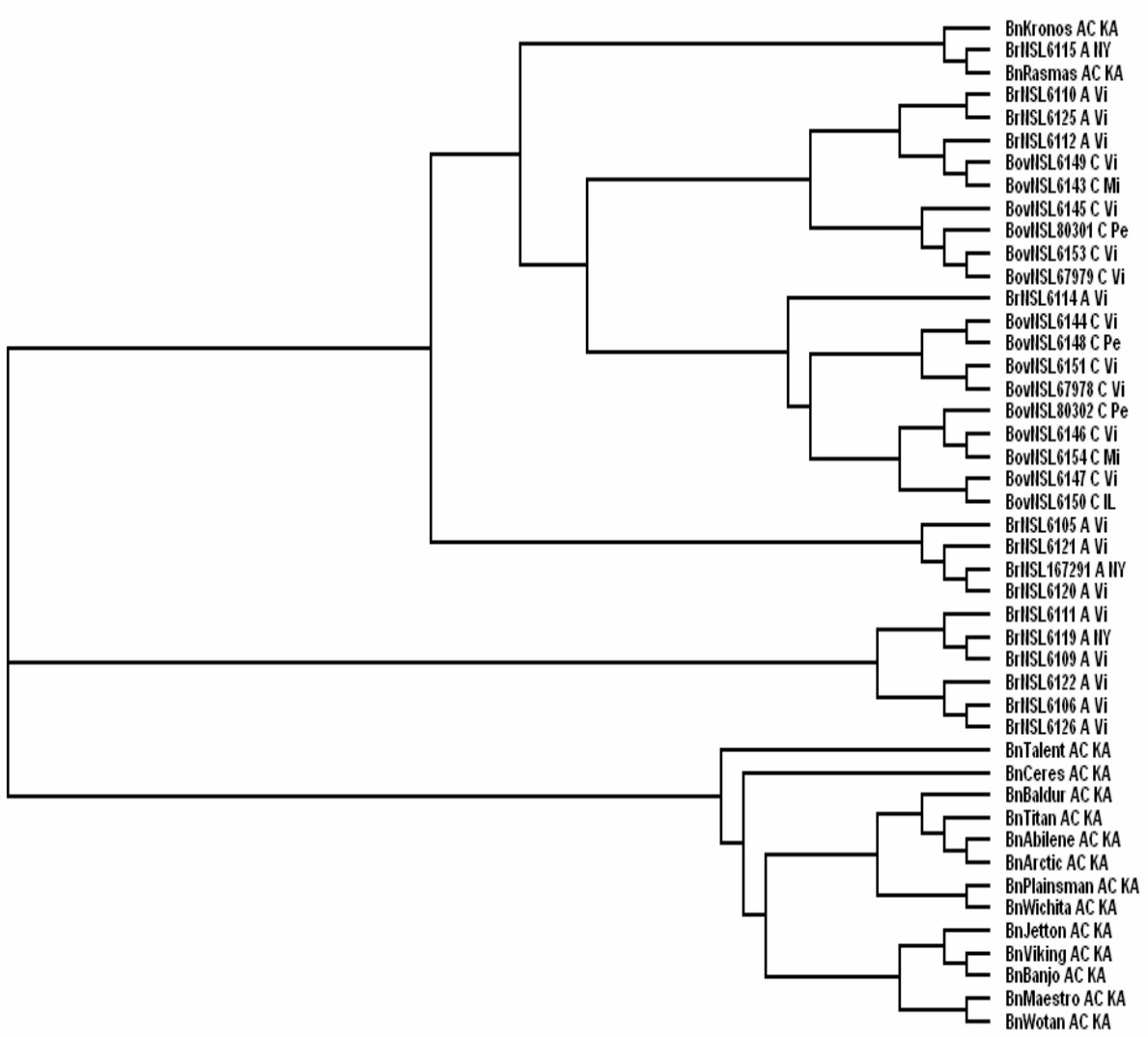

Figure 1. UPGMA cluster showing the genetic relationships among three Brassica species (B. rapa, B. oleracea, B. napus) at 13 RAPD loci associated with blackleg resistance. The first two letters in the sample labels refer to the taxonomic abbreviations (eg., Bo- Brassica oleracea; Br- Brassica rapa; Bn- Brassica napus), followed by the Accession number. The genomic grouping (A, C or AC) precedes the abbreviation for sample

NSL6105 (*B. rapa*), NSL6146 (*B. oleraceae*) and NSL6115 (*B. rapa*) were supported as a clade. However, in the context of the three accessions considered together, accessions NSL6105 and NSL6146 do not (or are not believed to) share a most recent common ancestor (MRCA). Several other cases of paraphyly are notable from the dendrogram, including the basal node. In general, cultivars and accessions that share a genome classification were expected to show the closest phylogenetic relationship.

Paraphyletic relationships observed in this study can most parsimoniously be explained by selection at the RAPD loci. The RAPD markers assayed in this study are associated with blackleg resistance. As such, they are expected to be under functional constraint, such that a phylogeny reconstructed at these loci reflects both functional as well as taxonomic relationships. Under this scheme, taxonomic species may not form a clade. However, other causes, both statistical and genealogical, can explain the paraphyly observed. Common statistical causes include inadequate or non-judicious sampling, inappropriate tree reconstruction methods, and inadequate phylogenetic characters. Incongruence among trees that has its origin in genealogical discordance includes: Hybridization (McKinnon et al. 1999; Avise, 2000), homoplasy (McCracken and Sorenson, 2005), polyploidy and paralogy (Ochieng et al., 2007b). Polyploidy is widely reported in plant taxa; and one of the species analysed here (*B. napus*) is a tetraploid. Polyploid formation following hybridization is plausible since

species within *Brassica* are reported to readily hybridize (Brun et al., 2001). Inappropriate tree reconstruction methods would pose a problem only in the basal relationships rather than in terminal taxa; the paraphyly observed in this study applied mostly to terminal taxa. This study recovered sufficient phylogenetic characters to separate accessions of the same species, making character deficiency an unlikely cause of incongruence between species tree and gene tree in these species. Similarly, inadequate sampling can be discounted because it is expected to lead to type-2 error (where cryptic paraphyly exists).

Divergent and potentially susceptible accessions were identified

The evolutionary histories of genes might retain signatures of functional demands to which they have been subjected, so that phylogenetic analyses can elucidate functional relationships within living cells (Ochieng et al., 2007a). Eleven genotypes of *B. napus* (AC genome; Abilene, Baldur, Banjo, Ceres, Kronos, Plainsman, Talent, Titan, Viking, Wotan, and Wichita) in this study have shown resistance in greenhouse and field experiments (Ananga et al., 2006). In this study, 13 out of the 15 accessions formed a clade, with a trichotomous basal topology showing a sister relationship with *B. oleracea* and *B. rapa* (Figure 1). These two accessions were evolutionarily so divergent from the main *B. napus* clade that we can reasonably conclude that they are susceptible to blackleg. The two accessions, Kronos and Rasmus (Sample 22 and 25; Table 1), were clustered outside of this clade. In a greenhouse study performed by Ananga et al., (2006), Rasmus and kronos were classified among the susceptible cultivars. Similarly, three accessions and accession groups belonging to *B. rapa* were identified from the phylogram: The most divergent group was NSL6115, NSL60110, NSL6125, NSL6112 and NSL6114. The subclade comprising NSL6105 was, however, not significantly divergent from the main *B. rapa* clade (one of the clades in the trichotomy; including NSL6111 and NSL6112).

In conclusion, this study has shown that *Brassica* accessions belonging to three species (*B. rapa*, *B. napus* and *B. oleracea* var. *virids*) showed both homology and functional relationships when their phylogeny was reconstructed using RAPD markers associated with resistance to a fungal pathogen (blackleg). Consequently, two potentially susceptible accessions can be identified from the phylogenetic trees, authenticating the utility of phylogenetic methods in predicting response to blackleg disease in *B. napus*. The high polymorphic information content (PIC) and number of phylogenetically informative bands show RAPD as a useful tool for phylogenetic reconstruction, quantification of genetic diversity for conservation, cultivar classification and molecular breeding.

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REFERENCES

Ananga A, Cebert E, Soliman K, Kantety R, Pacumbaba RP, Konan K (2006). RAPD markers associated with resistance to blackleg disease in Brassica species. Afr. J. Biotechnol. 5(22): 2041-2048.

Ansan-Melayah D, Balesdent HM, Buee M, Rouxel T (1995). Genetic characterization of *AvrLml,* the first avirulence gene of *Leptosphaeria maculans.* Phytopathology 85: 1525-1529.

Ansan-Melayah D, Balesdent HM, Delourme R, Pilet LM, Tanguy X, Renard M, Rouxel T (1997a). Gene-for-gene interactions in the *Leptosphaeria maculans-Brassica napus* pathosystem. Acta Horticulturae 459: 329-334.

Avise JC (2000). The history and formation of species Harvard University Press, Cambridge, Massachusets.

Banga SS (1993). Heterosis and its utilization. In: Labana KS, Banga SS, Banga SK (eds). Breeding oilseed *Brassicas*. Springer-Verlag, Berlin, Heidelberg, New York. pp. 2-38.

Botstein D, White RL, SkolnickMand David R (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32: 314-331.

Brummit RK (2002). How to chop up a tree. Taxon. 51: 31-41.

Brun H, Ruer D, Levivier S, Somda I, Renard M, Chevre MA (2001). Presence in *Leptosphaeria maculans* populations of isolates virulent on resistance introgressed into *Brassica napus* from the *B. nigra* B genome. Plant Pathol. *5*0: 69-74.

Chevre AM, Barret P, Eber F, Dupuy P, Brun H, Tanguy X, Renard M (1997). Selection of stable *Brassica napus-B. juncea* recombinant lines resistant to blackleg *(Leptosphaeria maculans).* 1. Identification of molecular markers, chromosomal and genomic origin of the introgression. Theor. Appl. Genet. 95: 1104-1111.

Edwards K, Johnstone C, Thompson C (1991). A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucl. Acids Res. 19: 1349.

Garcia-Mas J, Oliver M, Gomez-Paniagua H, de Vicente MC (2000). Comparing AFLP, RAPD and RFLP markers for measuring genetic diversity in melon. Theor. Appl. Genet. 101: 860-864.

Hongtrakul V, Huestis GM, Knapp SJ (1977). Amplified fragment length polymorphisms as a tool for DNA fingerprinting sunflower germplasm: genetic diversity among oilseed inbred lines. Theor. Appl. Genet. 95: 400-407.

Inaba R, Nishio T (2002). Phylogenetic analysis of Brassicaceae based on the nucleotide sequences of the S-locus related gene, *SLRI*. Theor. Appl. Genet. 105: 1159-1165.

Lazaro A, Aguinagalde I (1998). Genetic diversity in *Brassica oleracea* L. (cruciferae) and wild relatives (2n=18) using RAPD markers. Ann. Bot. 82: 829-833.

Mailer RJ, Scarth R, Fristensky B (1994). Discrimination among cultivars of rapeseed (*Brassica napus* L.) using DNA polymorphisms amplified from arbitrary primers. Theor. Appl. Genet. 87: 697-704.

McCracken KG, Sorenson MD (2005). Is Homoplasy or Lineage Sorting the Source of Incongruent mtDNA and Nuclear Gene Trees in the Stiff-Tailed Ducks (Nomonyx-Oxyura)? Syst. Biol. 54: 35-55.

McKinnon GE, Steane DA, Potts BM, Vaillancourt RE (1999). Incongruence between chloroplast and species phylogenies in *Eucalyptus subgenus* Monocalyptus (Myrtaceae). Am. J. Bot. 86: 1038-1046.

Ochieng JW, Muigai WT, Ude GN (2007a). Phylogenetics in plant biotechnology: principles, obstacles and opportunities for the resource poor. Afr. J. Biotechnol. 6: 639-649.

Ochieng JW, Henry RJ, Baverstock PR, Steane DA, and Shepherd M (2007b). Nuclear Ribosomal Pseudogenes resolve a corroborated Monophyly of the eucalypt genus *Corymbia* despite misleading hypotheses at functional ITS paralogs. Mol. Phylogen. Evo. 44: 752-764.

Rimmer SR, Buchwaldt L (1995). Diseases. In: *Brassica Oilseeds.* (eds.) Kimber DS, Mcgregor DI. CAB International, Wallingford, pp 65-92.

Rimmer S, Scarth R, McVetty P, Woods D, Gugel R, Séguin-Swartz G (1995). Breeding for resistance to blackleg (stem canker) in Western Canada. Blackleg Newsl. 5: 11-15.

Roy NN (1984). Interspecific transfer of *Brassica juncea* - type high blackleg resistance to *Brassica napus.* Euphytica 33: 295-303.

Salisbury PA, Ballinger JD, Wratten N, Plummer MK, Howlett JB (1995). Blackleg disease on oilseed Brassicas in Australia - a Review. Austr. J. Exp. Agric. 3(5): 665-674.

Snowdon RJ, Friedt W (2004). Molecular markers in Brassica oilseed breeding: current status and future possibilities. Plant Breed. 123: 1-8.

U N (1935). Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jpn. J. Bot. 7: 389-452.