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GENETIC DIVERSITY IN SEED LIPID COMPOSITION OF SOME WILD RADISH (*RAPHANUS RAPHANISTRUM*) POPULATIONS UNVEIL ITS POTENTIAL UTILITY FOR GENETIC IMPROVEMENT OF OILSEEDS

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ABSTRACT

Background Genetic variation in wild populations can be an important source for improving plant growth, yield and quality characteristics. This study was conducted to identify genetic variations in the oil contents and fatty acid composition of wild radish (*Raphanus raphanistrum*) populations, so that breeders may be able to select wild genetic resources for improving yield and quality of related oilseeds.

Methodology The fatty acid composition of lipids was measured in wild radish populations from Merredin, Old Nukarni, and Mullewa in the Western Australia and Denmark in lower south-west.

Results The total lipid content of whole seeds ranged from 38.7 to 42.2%. Within site, variations accounted for 80% and between sites variations were 20% of the total variations, and fatty acids ranged from 8.9 to 10.3%. The results indicated that within site, variations were much greater than between sites variations, hence probably it was necessary to go beyond Australia to fully explore genetic variations in oil content of wild radish. Furthermore, wild radish oil contained commercially important fatty acids: palmitic, stearic, oleic, linoleic, linolenic and erucic acids showing promising potential to be used as diesel, lubricating oils and surface coatings.

Conclusion The genetic variations that existed within and between populations of wild radish could be exploited to induce disease resistance, drought tolerance, resistance to pod shattering and uniform flowering in related oilseeds through breeding.

INTRODUCTION

Wild radish (*Raphanus raphanistrum*) is a widespread invasive plant of many agro-ecological regions throughout the globe (Goggin et al. 2019), including the crops of southern Australia (Piggin et al. 1978). Genetic variations in wild populations have been analyzed in a number of ways (González-Jara et al. 2011). The majority of studies used isozymes (Layton and Ganders 1984; Hassan et al. 2013), morphological and the physiological life history traits (Cocks et al. 1982; Bennett 1997; Dunbabin and Cocks 1999) and DNA-based molecular markers (Bennett and Mathews 2003; Lindqvist-Kreuze et al. 2003; Park et al. 2003). However, most members of the Brassicaceae contain seed oils, and *Raphanus* spp. are no exception (Kimber and McGregor 1995). While the seed oils are potentially of economic significance, they may also be used to assess genetic variations in wild populations. For example, there are some variations in the erucic acid content of *Brassica napus* which has enabled the selection of low erucic

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acid lines of oil seed rape (Nuttall and Moulin 1992; Salisbury and Ballinger 1995). The widespread distribution and associated genetic variations of wild radish in Western Australia, suggest that improvement in oil content and composition is possible. However, wild radish has only been present in the region for about 168 years. Variations, if present, will suggest either that there were many introductions with different fatty acid content or that natural selection is strongly favoured fatty acid patterns in particular locations.

Wild radish is a member of the Brassicaceae, a family that has contributed a number of important crops. The most important in southern Australia is canola which produces seed with <2% erucic acid and <40 mol/l of total glucosinolates (Nuttall and Moulin 1992; Salisbury and Ballinger 1995). These traits were derived from the Canadian cultivar "Zephyr" (low erucic acid) and the European cultivar "Erglu" (low glucosinolates) (Roy 1984). Because of its quality, canola oil is recognized as nutritionally superior to most other edible oils. In addition to its low erucic acid, canola is characterized by low levels of saturated fatty acids (less than 6% palmitic acid) and relatively high levels of oleic (55-60%) and linolenic acids (8-10%) (McDonald 1995). Wild radish is an important economic weed in canola, partly because it raises the erucic acid levels in canola oil and hence reduces oil quality.

Although there are many reasons why wild radish would be difficult to domesticate, the presence of useful oils may stimulate interest in either the use of wild radish in breeding programs for other members of the Brassicaceae or domestication of the species itself. It is therefore useful to know more of the pattern of fatty acids in this species and especially any genetic variation that occurs. This study describes the fatty acid content of the oils of wild radish collected from four contrasting sites in south-western Australia. Total oil content were measured in plants grown in Perth and the fatty acid content of the oils were determined using gas chromatography. The results were related to variation in other aspects of the south-western populations and discussed in terms of their evolutionary and economic significance. In this work, the hypotheses tested were; 1) wild radish populations exhibit genetic variations in the quality of seed oils, and 2) the yield and quality of oils and their fatty acids will provide future useful genetic material, either in their own right or in the genetic improvement of related species.

MATERIALS AND METHODS

Wild radish seeds were collected from Merredin (31^0 22'S, 118^0 32'E), Old Nukarni (31^0 23'S, 118^0 15'E), Mullewa (28^0 33'S, 115^0 29'E) and Denmark (34^0 58'S, 117^0 39'E) (Figure 1). Maximum and minimum January and July temperatures and annual rainfall at each collection site are shown in Table 1. A description of the plant material is in Table 2.

Lipids from the whole seeds were extracted using a Soxhlet apparatus (Gunstone 1996). Whole seeds (5 g) were ground into a fine powder with a coffee grinder. Three replicates (1 g samples) of each population were extracted for six hours with 20 ml of hexane (99.5%). The fatty acid composition was determined by gas chromatography (GC) on the liquid samples by converting the extracted liquids to fatty acid methyl esters (Alonso et al. 1997). A known amount of each lipid extract (15-30 mg) was dissolved in 4 ml hexane in a stoppered tube. One ml of methanolic potassium hydroxide (2 M) was then added as a trans-esterification agent. After thorough mixing, the solution stood for 20 minutes, after which the hexane layer was removed and transferred into glass vials. GC separation of the fatty acid methyl esters (FAMES) was performed on a GC-17A V3 (SHIMADZU, Japan) equipped with flame-ionization detector and flow splitter. The GC conditions are described in Table 3.

One µl of the hexane fraction was injected into the apparatus. For quantitative analysis, non-adecanoic acid methyl ester (C19) was used to establish calibration curves as an external standard. Each testing run had its own calibration curve. The peaks had a clear base line and were identified on the basis of their retention times and standards.

Data analysis

In order to determine variation between and within sites for the total lipid content and for each fatty acid, a general analysis of variance was performed separately on each variable. The effect of site environment on fatty acid composition was assessed using principal component analysis. Plants were grouped according to the site clusters and the mean PC1 score plotted against the mean PC2 score for each group. The fatty acid composition of wild radish was compared with a number of oil seed crops using principal component analysis in a similar way.

RESULTS

The total lipid content of the whole seeds ranged from 38.7% at Nukarni to 42.2% at Merredin (Table 4). Most of the variations (80%) occurred within populations. The mean fatty acid profile of all plants tested is shown in Figure 2. The perusal of data showed that the dominant fatty acids are erucic acid C22.0), oleic acid (C18.1), linoleic acid (C18.2), linolenic acid (C18.3) and eicosenic acid (C20.1).

Table 1 Mean maximum and minimum January and July temperatures (°C) and mean annual rainfall (mm) at Merredin, Nukarni, Mullewa and Denmark

Site locations	January maximum	January minimum	July maximum	July minimum	Mean annual rainfall
Merredin	34	17	16	5	314
Nukarni	34	18	16	6	328
Mullewa	37	19	19	7	340
Denmark	25	14	17	7	1001

Table 2 Mean seed and pod weight, number of pods and the range of pod widths and lengths of the plants collected from Merredin, Mullewa, Nukarni and Denmark when grown in a common garden at Shenton Park near Perth

Sample ID	Seed weight (g 100 seed^{-1})	Pod weight (g 10 pods^{-1})	Number of pod segments	Pod width (mm)	Pod length (mm)
Merredin	0.70	1.88	3 to 10	0.1 to 0.5	23 – 61
Mullewa	1.29	3.49	1 to 12	0.2 to 0.7	32 – 79
Nukarni	0.46	1.05	2 to 7	0.1 to 0.3	20 – 56
Denmark	0.47	1.15	2 to 6	0.1 to 0.4	19 – 51

Table 3 Chromatographic conditions used in analysis of wild radish fatty acid methyl esters by GC with a BPX-70 (50mx 0.22 mm) capillary.

Parameter	Setting
Column head pressure	200 Kpa
Split flow ratio	35
Oven temperature	
Initial temperature	170^{0}C
Initial time	35 min
Temperature rate	5^{0}C/min
Final temperature	220^{0}C
Final time	5 min
Injector temperature	250^{0}C
Detector temperature	285^{0}C
Carrier gas	Helium
Detector gas	Hydrogen

Table 4 Total lipid content and the content of each constituent fatty acid of the Merredin, Mullewa, Nukarni and Denmark populations.

Lipids and fatty acids	Merredin	Mullewa	Nukarni	Denmark	CV (%)	% Variation Between Sites
Total lipids	42.2	41.9	38.7	41.2	6.9	20.4*
Palmitic (C16:0)	5.51	4.93	5.55	5.35	11.6	7.7 n.s
Stearic (C18:0)	2.38	2.06	2.23	1.72	13.6	44.4***
Oleic (C18:1) n9	17.39	14.83	15.24	17.05	12.5	27.1***
Linoleic (C18:2)	11.65	10.25	11.73	11.58	12.2	8.2**
Linolenic(C18:3)	12.78	12.63	13.19	13.45	12.2	7.0 n.s
Arachidic (C20:0)	1.3	1.03	1.33	0.87	20.7	38.1***
Eicosenoic (C20:1)	10.05	9.35	9.53	9.11	12.8	7.3 n.s
Behenic (C22:0)	1.06	1.18	1.17	0.94	16.8	18.5***
Erucic (C22:1)	33.21	36.28	36.12	36.24	12.2	8.1*
Others	3.62	3.14	3.91	3.64	90.2	0.24 n.s
Saturated	10.25	9.2	10.28	8.88		
Unsaturated	85.08	83.34	85.81	87.43		
Saturated/Unsaturated	1:8.30	1:9.06	1:8.35	1:9.85		

The table also shows the amount of variation accounted for between populations and the coefficient of variation (CV %) of each fatty acid across all populations. Significance of the difference between populations is P<0.001 (***), P<0.005 (**), P<0.05 (*).

For each site, the fatty acid profiles are shown in Table 4. The results indicated that the fatty acid profiles from all four sites are broadly similar, although 6 of the 9 main fatty acids differed significantly between sites. Also the fatty acids were composed of approximately 8.9 to 10.3% saturated and 83.3 to 87.4% unsaturated. The ratio of saturated/unsaturated fatty acids ranged 1:83 to 1:10.

Stearic acid (44%) showed the most between-sites variations, while arachidic (38%), oleic (27%), linolenic (7%) and eicosenic (7%) showed the least variations between sites. Within-site variations were significantly greater than between-sites variations. The acids with the greatest within-site variations were those with the least between-sites variations: linolenic (93%) and eicosenoic (93%) acids. Stearic (56%), arachidic (62%) and oleic (73%) acids displayed the least within-site variations.

Component loadings arising from the principal component analysis are shown in Table 5. The first PC accounted for 40.5% of all variations and the second for 23.6%. In Figure 3, populations were grouped according to collection sites and plotted against their average scores. The Figure indicated that the populations differed in respect to PC2 (low values of PC2 accompany high values of behenic, stearic and arachidic acids) with populations from Denmark and Mullewa having strikingly similar fatty acid profiles. Within the range recorded here, Merredin and Nukarni populations tended to have lower values for the three fatty acids than do the Mullewa and Denmark populations. PC1 comprised of a set of fatty acids, which did not appear to differ significantly between collection sites. In particular, the Mullewa population exhibited a great deal of variability for this set of fatty acid parameters.

DISCUSSION

This study explored the use of lipid and fatty acid analysis to study genetic variation in Western Australian populations of wild radish. Although the populations were collected from different sites with very different environments, their seed oils and fatty acids compositions were markedly similar. All populations of wild radish seed in this study contained high levels of erucic, oleic, linoleic and linolenic acids. There were significant differences between populations in fatty acid composition, although they were relatively minor. The data also showed that within site variations are much greater than between sites variations, suggesting that there has been very little evolution in the fatty acid content and composition of this species since its introduction to Western Australia.

This result was not surprising because, in out-breeding species such as wild radish, strong selection pressure was needed for populations to develop differences in the short time that wild radish has been in Western Australia. For example Hamrick et al. (1979) in reviewing the literature of plant species with different breeding systems, found that for most traits, outbreeding species have a greater proportion of their variation within populations and less between populations. The similarity in the seed oils of wild radish populations differed from some studies of naturally occurring oils found in the literature. For example, in the Lamiaceae, genetic variation is widespread in *Thymus vulgaris*, *Rosmarinus officinalus* and many other species (Khalil et al. 2012). In particular, in *Cunila galioides*, it was found that oils and some of their constituents are under genetic control and showed ecotypic differentiation, with certain chemotypes restricted to particular geographical areas. In the Brassicaceae, similar variation occurred in the composition of oils, with oleic and erucic acids varying widely in *Brassica carinata*.

In the light of this evidence it was likely that more variations might be found in wild radish if further populations were sampled from different localities. The fact that only four populations were sampled, all from Western Australia, severely limited the opportunities for genetic variation to be expressed. If populations from throughout Australia were sampled the opportunities for finding variation would be greatly increased. However, given that we know of no reason why there would be an evolutionary advantage for different oil compositions and that there is no evidence of separate introductions with different fatty acid compositions, it is likely that the species has been in Australia for an insufficient period for genetic drift and mutation to have developed distinct chemotypes. Therefore, it would probably be necessary to go beyond Australia if the genetic variation in oil content of wild radish is to be fully explored. The results also suggested that, as far as oils and their composition was concerned, wild radish, when introduced to Western Australia, showed little genetic variation. If we assume that there were multiple introductions of wild radish and that, for example, the Nukarni population represented a different introduction from the other three sites, the lack of variation here suggested that there may not be significant variation in the Australian or indeed the World population. Such a conclusion however, would need confirmation from further, more widely based surveyed. A comparison with other species containing oil is interesting because of the possible commercial exploitation of wild radish. Wild radish oil contained six commercially important fatty acids: palmitic, stearic, oleic, linoleic, linolenic and erucic

Table 5. Component loadings for plant fatty acids in the principal component analysis, as well as the amount of variation accounted for by the first and second principle components.

Fatty acids	Latent Vectors	
	PC1	PC1
% Variation	40.5	23.6
Palmitic (c16:0)	0.464	0.111
Stearic (c18:0)	0.371	-0.419
Oleic (c18:1)	0.286	0.350
Linoleic (c18:2)	0.430	0.173
Linolenic (c18:3)	0.195	0.308
Arachidic (c20:0)	0.394	-0.419
Eicosenic (c20:1)	0.427	0.060
Behenic (c22:0)	0.000	-0.608
Erucic (c22:1)	0.073	0.126

Table 6 Component loading for plant fatty acid composition in the principle component analysis of the fatty acid content of nine oil crops and four populations of wild radish, as well as the amount of variation accounted for by the first and second principle components

Plant fatty acid compositions	Latent Vectors	
	PC1	PC2
% variation	34.1	16.9
Arachidic (c20:0)	0.41	-0.24
Behenic (c22:0)	0.36	-0.25
Eicosenoic (c20:1)	0.10	0.55
Erucic (c22:1)	0.43	0.05
Lignoceric (c24:0)	0.08	0.11
Linoleic (c18:2)	-0.17	-0.16
Linolenic (c18:3)	0.04	-0.04
Oleic (c18:1)	-0.30	-0.37
Palmitic (c16:0)	-0.31	-0.11
Stearic (c18:0)	-0.26	-0.28
Others	0.27	-0.07

acids. With its high erucic acid content it could not be used for food purposes; however, it could be used for non-food purposes, such as diesel fuel, lubricating oils and surface coatings. Its use for food would be limited to food condiments like mustard seed. Principal component analysis was performed on the fatty acid composition of different vegetable oils to provide comparisons with wild radish oil. Data for these oils were extracted from published sources (AOCS 1996). Table 6 showed the component loadings for individual fatty acids of the different oils and the amount of variation accounted for by PC1 (34% of the total) and PC2 (17%). The Figure 4 showed clearly that the wild radish oils were similar to mustard seed oils, but because of its high erucic acid, less so to other common vegetable oils.

This conclusion was supported by the fact that canola oil, obtained from modified rapeseed, separated in the analysis from wild radish mainly because of its low erucic acid content, highlighting the problem that contamination with wild radish could cause unacceptably high levels of erucic acid in commercial canola oil. The economic importance of commercial oil crops such as canola depends not only on their oil contents but also on the quality and amount of the protein meal remaining after oil extraction. This aspect was not examined during this study. However, it is unlikely that wild radish protein would be significantly inferior to canola protein. Further work in this area is needed if a full understanding of the commercial potential of wild radish is to be realised. Of course, the possession of some interesting traits is hardly likely, of itself, to lead to the domestication of a weedy species such as wild radish. However, the genetic variation that existed within and between populations suggested that selections of wild radish for disease resistance, drought tolerance, resistance to pod shattering, and uniform flowering time may be possible through plant breeding. The domestication of wild radish might then become possible for niches where other oil seeds are unreliable because of low rainfall or

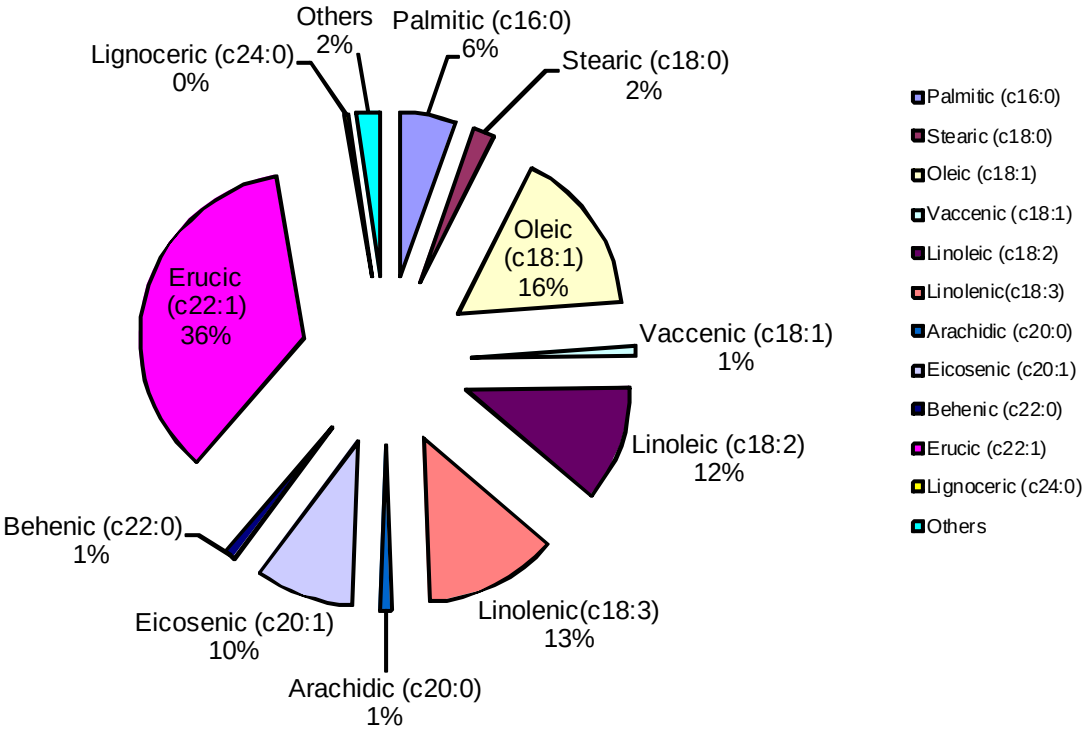

Figure 2 Mean values of fatty acid composition of wild radish seeds sampled from Merredin, Mullewa, Nukarni and Denmark in south-western Australia.

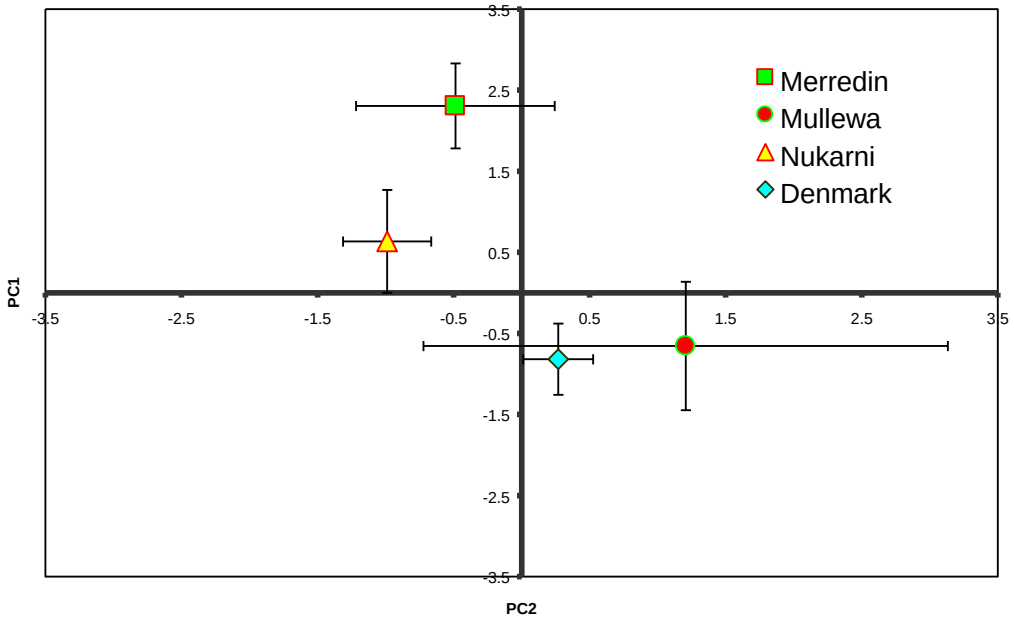

Figure 3 Principal component analysis showing the relationship between plant fatty acids and environment. Points represent the mean of PC1 and PC2 scores for all plants within each site. Error bars ± I s.e.

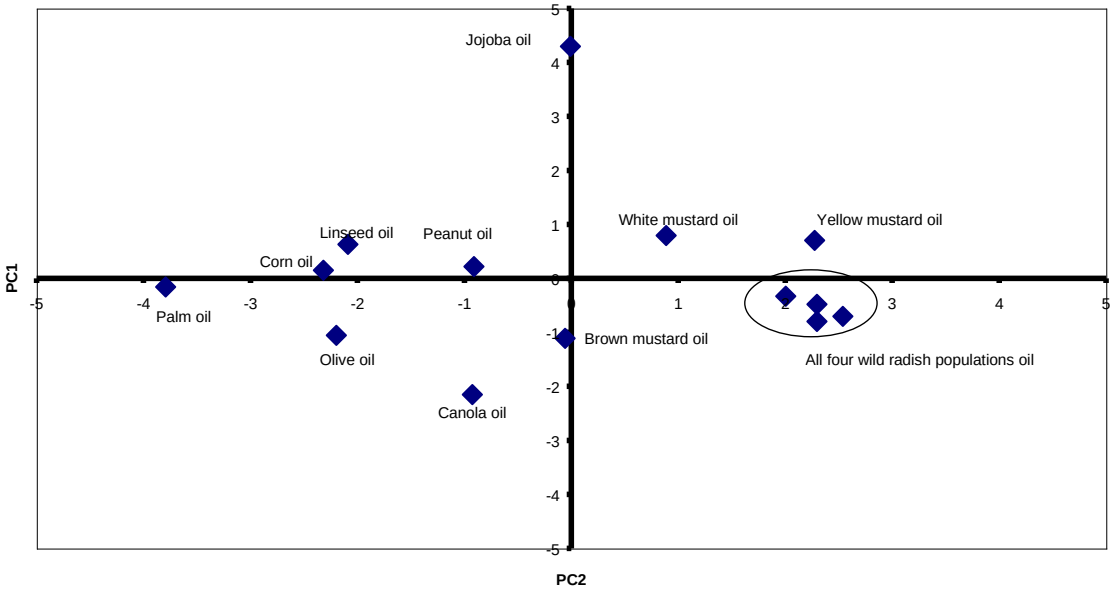

Figure 4 Principal component analysis showing the relative profiles of fatty acids for different vegetable oils. Points represent the mean of principal component scores for all crops (AOCS) 1996)

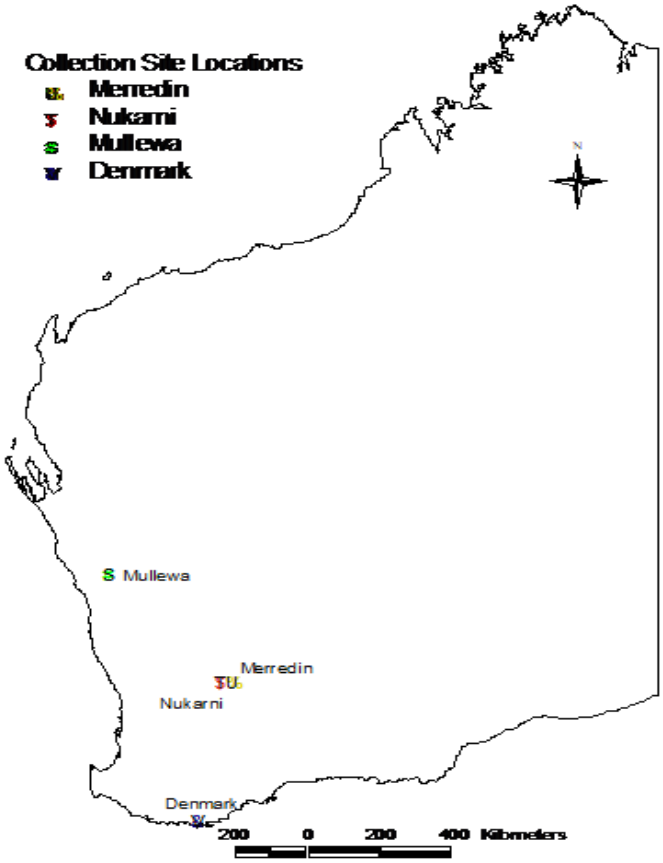

Figure 1 Wild radish population collection sites around Western Australia.

inhospitable soil. Perhaps more likely is the use of these traits in wild radish for wide crosses with closely related species such as canola or mustard.

Outcrossing and self-fertilization are common within the genus *Brassica* (Yamamoto and Nishio 2014). Canola is known to be predominantly self-fertile with the potential of about 30% outcrossing (Williams 1986; Rakow and Woods 1987), which nevertheless resulted in the free and widespread dispersal of genes. Chevre et al. (1994) demonstrated that hybrids could be produced between wild radish and canola. Spontaneous hybridization between male-sterile canola and wild radish generated 3,734 hybrid seeds/m^2. High chromosome pairing was observed and the hybrid pollen fertility was measured at 0-30% (Eber et al. 1994). Further research on the spontaneous outcrossing and seed production between various male-sterile and transgenic lines of canola with wild radish led to the discovery that the seeds produced showed size dimorphism (Baranger et al. 1995). The use of wild radish genes in canola is therefore possible and where useful genes can be identified desirable. However, there is a down side to the relatively straightforward hybridisation between these species. Triazine-resistant canola varieties are commonly used in southern Australian farming systems and other herbicide resistant cultivars are likely in the near future through genetic modification. Given that spontaneous hybridisation is possible, it is likely that wild radish will receive trans-genes and produce hybrids. Herbicide susceptible wild radish is a target for control within the herbicide-resistant crop, but the probability of hybridisation with canola and the possibility that the resulting hybrids may be fertile runs the risk of transferring the resistance gene to wild radish.

CONCLUSIONS

The results led to conclude that within site genetic variations in seed oil contents and fatty acid composition of wild radish populations were much greater than between sites variations, suggesting that it would probably be necessary to go beyond Australia if the genetic variation in oil content of wild radish was to be fully explored. The genetic variations identified within and between populations could be exploited to breed oilseed crops having uniform flowering, tolerant to drought and resistant to diseases and pod shattering. Wild radish oil contains six commercially important fatty acids (i.e. palmitic, stearic, oleic, linoleic, linolenic and erucic acids) nevertheless its high erucic acid contents makes it unfit for food purposes; however, it could be used as diesel fuel, lubricating oils and surface coatings.

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