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INTERSPECIFIC TRANSFER OF *BRASSICA JUNCEA*-TYPE HIGH BLACKLEG RESISTANCE TO *BRASSICA NAPUS*

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INDEX WORDS

Brassica napus, rapeseed, *Brassica juncea*, *Leptosphaeria maculans*, blackleg resistance, interspecific cross, gene transfer, polygenic resistance, seedling and adult resistance.

SUMMARY

Complete resistance to *Leptosphaeria maculans*, the cause of blackleg of oilseed rape (*Brassica napus*), was transferred from *B. juncea* to *B. napus* through an interspecific cross. *B. juncea*-type complete resistance (JR) was recognized first in one F_3 progeny (Onap^{JR}) by the absence of leaf-lesions on seedlings and canker-free adult plants. The commercially important characters of *B. napus* were retained in advanced lines of Onap^{JR}, which combined JR with low erucic acid levels (< 0.5%), high seed yield and variable maturity dates.

JR appeared to be inherited as a major gene or genes. Segregation for resistance and susceptibility continued to occur during later generations of selection of Onap^{JR}. JR was readily transferred from Onap^{JR} to other suitable *B. napus* cultivars or lines with partial resistance to blackleg and resulted in highly vigorous early generation selections adapted to cold, wet situations along with complete resistance to blackleg.

INTRODUCTION

Blackleg (caused by *Leptosphaeria maculans* (DESM.) CES et DE NOT.) is a serious disease of rapeseed (*Brassica napus* L.) and causes extensive crop loss in many parts of the world including Western Australia (McGEE & PETRIE, 1979). Breeding for blackleg resistance in Western Australia has been top priority of the rapeseed breeding programme (ROY & REEVES, 1975)). Until recently only partial resistance or adult resistance has been found within *B. napus*. Partially resistant seedlings get leaf-lesions caused by the ascospores (primary infection) and later pycnidiospores (secondary infection) discharged from the infected leaves. Thus there is some extent of damage to the leaf photosynthetic area. Later, in the susceptible plants, the infection spreads from the leaf to the base of the stem forming cankers. However, in cultivars with adult plant resistance, 60–80% of the plants remain canker-free. Therefore the long-term breeding strategy has been to look for additional sources of resistance genes from an allied species (such as *B. juncea*) which shows complete resistance in both seedling and adult. Transfer of such resistance to *B. napus* through interspecific crosses though rare is possible (ROY, 1978a, 1983). In this paper results are presented of investigations carried out with a *B. napus* selection (Onap^{JR}) with low erucic acid and *B.*

juncea-type high blackleg resistance (JR) developed from a successful *B. juncea* × *B. napus* cross.

MATERIALS AND METHODS

From a successful cross (75N313), of BJ168 (Indian *B. juncea* collection, completely resistant, high erucic acid levels) with Cresus-O-Precose (*B. napus*, susceptible, late maturing, low erucic acid levels) a single F_3 plant was selected in the Mt Barker disease nursery in 1977 showing the plant characters of *B. napus* along with *B. juncea* type complete resistance (JR). Subsequent tests confirmed its low erucic acid oil content. From this selection designated $Onap^{JR}$, large numbers of lines (F_4–F_7) have been developed during 1978–81 at the disease nursery and evaluated in preliminary yield tests in small plots (1.25 m × 10 m) with two replications.

In 1979 six F_5-lines selected from $Onap^{JR}$ were crossed with several *B. napus* lines known to have considerable field resistance (*B. napus*-type resistance, or NR) in order to transfer JR gene(s) into the *B. napus*-type resistance background. From this set of crosses, F_2 and F_3 populations were grown in 1980 and 1981 respectively in the disease nursery in 10 m rows. At least 1500–2000 F_2 progenies (8–10 rows) were grown from each cross and 400—800 plants (2–4 rows) for each F_3 line.

Plant stand, seedling growth and disease reaction were rated on basis of overall row-performance, as shown below:

GOOD: > 80% plant stand + good growth;
FAIR: > 40–80% plant stand + good to fair growth;
POOR: 10–40% plant stand, fair to poor growth;
NIL: < 10% plant stand.

In selecting for JR-type plants in the disease nursery rows, seedlings which showed no infection of cotyledons or early leaves were marked and followed to maturity. At the same time, there was good leaf-infection on the nearby susceptible control and NR-plant rows. At maturity single plants were pulled out and the crown/stem region examined. Only canker-free plants (rated R) were retained.

In case of NR-type plants, there was no scope of seedling selection based on leaf-lesions. Usually all the seedlings get leaf infection at some stage. The number and intensity of leaf-lesions on seedlings were variable depending on the presence of inoculum and stage of infection and were not likely related to intensity of crown canker development. In such type of resistance, irrespective of degree of leaf-infection, the resistant plants show no canker formation. Segregation for adult plant resistance was easy to recognise, and plants were rated canker-free (R), trace (T), and moderate to heavily cankered (= susceptible, S), (see Fig. 1). Only R- or T-rated plants were selected.

From advanced selections (F_6–F_8) grown in trial plots, the percentage of canker-free plants as worked out for each selection based on a number of random samples pulled out at any stage after flowering and before harvest. Such percentage rating was always compared with the control variety to take into account seasonal or site variation. The disease rating was based on the percentage of adult plants free from canker (R) on a scale of 1–10 as follows: 1 = 0–9%; 2 = 10–19%; ..., 9 = 80–89%, 10 = 90–100% (100% resistant).

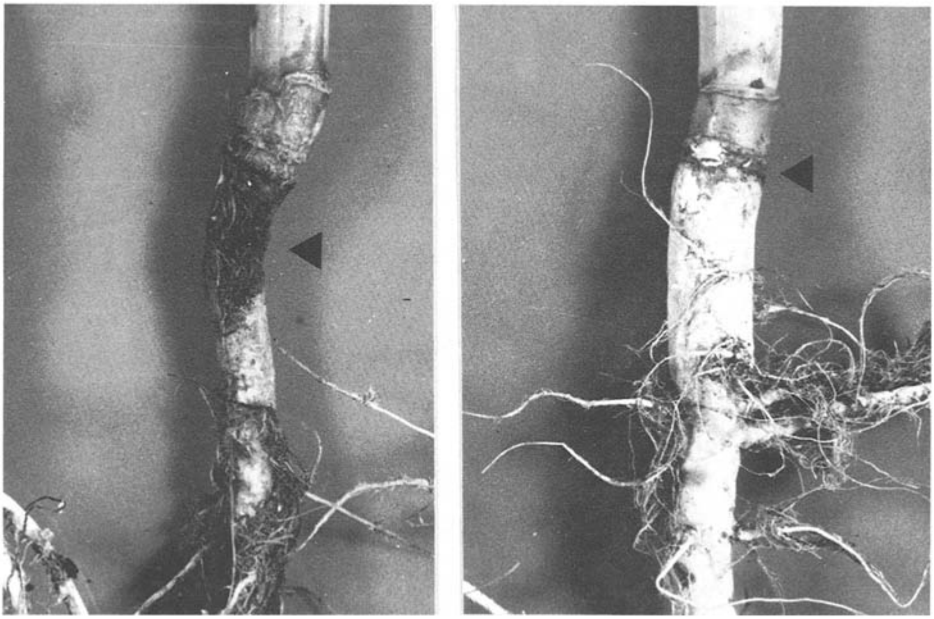

Fig. 1. Comparison of blackleg resistant and susceptible *B. napus*: Absence or presence of cankers at crown. Left: Blackleg susceptible-heavily cankered in crown region; right: Blackleg resistant-canker-free.

Analysis of erucic acid levels in the seeds of individual plants was carried out by the Grain Quality Laboratory, Department of Agriculture, Western Australia using conventional methods.

RESULTS

Development of Onap^{JR}. In 1976 F_2 progenies from cross 75N313 were grown in a disease nursery and partially fertile and intermediate plant types were selected. The disease reaction, plant type and seed sterility of the segregating F_2 population of this cross was reported before (Roy, 1978a). Some of the selected progenies were JR-type plants (i.e. clean leaf or seedling resistance and no canker development in the adult plant). Other progenies were NR-type plants – in that seedling – leaves showed infection (lesions) but adult plants remained canker-free.

In the F_3 rows some lines of cross 75N313 originally selected for JR proved completely seed-sterile. In other rows some of the high-yielding *B. napus* plants were found to be susceptible to blackleg. However, a number of JR-type fertile plants with *B. napus* characters were also found segregating in a few rows. Most of them were high in erucic acid, very late in maturity and were not carried forward. One such resistant progeny (75N313-15) was identified with low erucic acid (designated Onap^{JR}). When grown the following year in the disease nursery, this line survived with a relatively healthy plant stand (Fig. 3) whereas the neighbouring rows of *B. napus* crosses (NR-type plants) were more or less wiped out, indicating the severity of the disease. Most of the progenies (85–90%) of Onap^{JR} were of the *B. napus* plant type, with heavy leaves

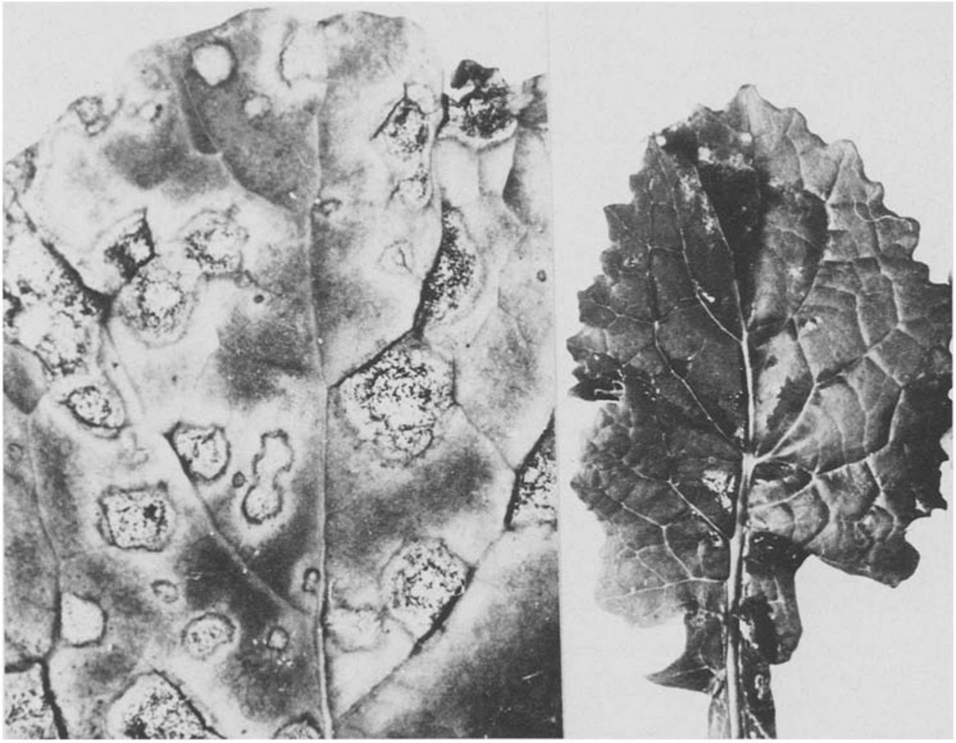

Fig. 2. Comparison of blackleg resistant and susceptible *B. napus*: severity of leaf infection on resistant (Onap^{JR}) and susceptible plants. Left: Susceptible-many lesions on leaf with pycnidia of *L. maculans*; right: Resistant-lesion-free. (Different magnification.)

but free of infection (JR-type plants) (Fig. 2). At harvest all these plants were found to be canker-free.

Onap^{JR} resistances. Table 1 gives the results of a preliminary trial conducted with some Onap^{JR} single plant selections (F_5) under high disease pressure in 1980. Compared to cross 75N70 (NR-type plants, brought from French and japanese *B. napus* sources), Onap^{JR} selections showed higher percentage of survival and clean plants (seedling + adult resistance) in spite of presence of heavy inoculum. Under that situation 65 (94%) selections of 75N70 were killed by infection at seedling emergence, the most vulnerable component against which NR-type resistance appears not to be effective. The other cross 76N219 of complex origin (*B. napus* × *B. juncea*), with NR-type adult plant resistance performed better than 75N70, though it was not better than Onap^{JR}.

It is clear that 44 (52%) Onap^{JR} selections became susceptible (Table 1) indicating loss of JR-gene(s) which may be through segregation or selection of plants that had escaped disease. Such segregation of JR-gene(s) became more apparent through observation of a large number of progenies (F_5–F_7) of Onap^{JR} lines over a three year period (1979–81) of continuous selection. Non-segregating lines could not be established and advanced generation lines (F_7) were found giving variable proportion of JR-type

Fig. 3. Survival of Onap^{JR}-line in disease nursery under severe disease condition that killed NR-type and susceptible *B. napus* lines in adjacent rows.

plants. Considering that the first year of selection of Onap^{JR} coincided with the F_3 generation of the cross, the F_7 generation included 5 years of continuous single-plant selection in Onap^{JR} for JR combined with other desirable characters. This is possibly due to selection favouring the heterozygote for JR genes. However, it has been possible to improve through selection growth and vigour, adaptability to cold wet situations, maturity (very early–late) and yield.

Yield potential of Onap^{JR}. In Table 2 results of a preliminary yield trial conducted under normal disease situation (i.e. grown near a previous crop but not on stubbles) indicate the high yield potential of 96 single plant selections (F_7) of Onap^{JR}. Five selections gave 30–50% higher yield than Wesroona, the best commercial variety with NR-type resistance. A number of selections were not only high yielding but also much earlier (– 10 days) compared to Wesroona, which is itself an early maturing variety. The high yield advantage of these selections could be partly due to less pod shattering compared to Wesroona.

Under the moderate disease pressure of this trial some of the Onap^{JR} selections showed 96–90% plants free-from canker. However most of the selections (Onap^{JR}) showed in some plants slight leaf-infection indicating late segregation for JR (seedling

Table 1. Disease performance of Onap^{JR} under high disease pressure. Mt Barker Research Station 1980 trial.

Cross (type of resistance)[2]	Number of resistant selections with stem canker rating (1–10)[1]						
	10	9	8	7	6	5–2	1
75N313 (Onap^{JR})	12	14	11	0	4	0	44
76N219 (*napus*^{NR})	1	5	7	3	1	0	36
75N70 (*napus*^{NR})	0	1	2	0	1	0	65

[1] 1 = 0–9% plants canker-free, 2 = 10–19% plants canker-free, ..., 9 = 80–89% plants canker-free, 10 = 90–100% plants canker-free.
[2] Onap^{JR} = *B. napus* with *B. juncea* type resistance (seedling and adult resistance); *napus*^{NR} = *B. napus*-source of resistance (adult resistance only).

Table 2. Performance of top 23 (24%) F_7 selections of Onap^{JR} in yield trial, 1982. Mt. Barker Research Station (under moderate disease pressure).

Yield (% Wesroona)	Number of selections					Total
	very early (15/9–17/9)	early (22/9–24/9)	med. early (25/9)	med. late (27/9)	late (2/10)	
130–155	1	2	–	2	–	5
110–120	3	1	1	1	1	7
101–109	4	4	–	3	–	11
Total	8	7	1	6	1	23

Wesroona Yield = 786 kg/ha.

Table 3. Number of selections made from different crosses under high disease pressure. Mt Barker Research Station, 1980 disease nursery.

Crosses		Plant stand	Resistant plants selected
type[1]	number		
Onap^{JR} (F_6)	1	Fair-good	1195
Onap^{JR} × *napus*^{NR} (F_2)	37	Fair-good	401
napus^{NR} × *napus*^{NR} (F_2–F_7)	53	Poor-nil	68

[1] Onap^{JR} = *B. napus* with *B. juncea* – type resistance (seedling + adult resistance); *napus*^{NR} = *B. napus* – source of resistance (adult resistance only).

resistance). The control Wesroona showed 85% plants free from canker.

These Onap^{JR} selections appeared to be normal in oil content (41–43%).

Transfer of JR-Gene(s) from Onap^{JR}. Attempts to transfer through crosses JR-gene(s) from Onap^{JR} to other high-yielding *B. napus* cultivars or lines with NR-type resistance genes appeared to be successful. In Table 3, plant stand and blackleg resistance of F_2 lines from 37 crosses with Onap^{JR} grown in the 1980 disease nursery are given. In these test rows were also grown 53 other crosses (F_2–F_7) among NR-type *B. napus* lines and included early generation progenies and advanced generation selections. Most of these NR × NR gave very poor stands or were wiped out indicating the high disease severity. However, the F_2 progenies of 37 crosses with Onap^{JR} gave fair to good plant stands. In a few specific Onap^{JR} × NR crosses, many of the progenies were without any leaf lesions (i.e. JR-type plants were dominant), in other crosses there was a variable degree of segregation. In all, 401 highly resistant single plants were selected, from Onap^{JR} × NR crosses. Only 68 plants (adult resistant) could be selected from the NR × NR group of crosses out of a few thousand rows planted. Most of the resistant selections (numbering 1195) came from Onap^{JR} (F_6) lines, some of which showed variable extent of segregation for JR.

From further observations based on a large number of populations involving Onap^{JR} crosses (F_3–F_4) grown in 1981–1982 and selections made from them (results not presented), indications were that it would be possible to build up increased resistance (seedling and adult) through specific combination of NR × Onap^{JR} crosses. The resultant progenies from such crosses appeared to be very good in growth, vigour and adaptability to cold wet environments.

DISCUSSION

Until recently with *B. napus*, a gene-complex conferring only partial resistance (seedling tolerance + adult resistance) was available. The sources of such resistance genes (designated NR) were some European and Japanese *B. napus* cultivars. The resistance mechanism appeared to be partial resistance, and likely to be polygenic (CARGEEG & THURLING, 1979). Using each of these sources of resistance, several blackleg resistant rapeseed varieties were developed recently (ROY, 1978b; ROY & POOLE, 1981; RENARD, personal communication). These varieties showed seedling infection (tolerance) but considerable adult plant resistance (60–80% of plants remained canker-free). By accumulating resistance genes from two or more sources some improvement in adult plant resistance was achieved in new crosses (viz. 75N70, 76N219) under our programme.

In the disease nursery at Mt Barker Research Station experimental lines were grown on the previous year's rape stubbles (residues). The cold wet start of the growing season always ensured heavy infection levels early in the growing season. The presence of natural inoculum in the stubbles close to the crown of plants incited not only blackleg but also other soil-borne diseases (*Sclerotinia* and damping off). Intensity of infection could be controlled to some extent by incorporating in the soil light to heavy applications of rape stubble. The resistant rape cultivars at present available showed considerable seedling tolerance provided infection started at post-emergence period, from cotyledon to early leaf development. Under heavy infection at germination or seedling – emergence stage, even the best of the partially resistant (NR) lines showed various extent of seedling mortality, indicated by reduced plant stand. In this situation JR-gene(s) from *B. juncea* offer more protection by making plants resistant at all stages of development from germination, emergence, seedling growth, to maturity. It was therefore considered a sound breeding strategy to try to introduce through interspecific crosses, if possible, such additional resistance gene(s) from *B. juncea* to *B. napus* without losing the existing polygenic resistance already accumulated in some of our varieties.

Though at F_1 stage some of the crosses between *B. juncea* and *B. napus* showed complete resistance (resistance dominant), in the early segregating generations there was practically no recovery of such *B. juncea*-type (JR) resistance among *B. napus*-type progenies. In most cases, all the progenies were eliminated at F_2 and thereafter by high seedling mortality, poor growth and seed sterility caused by chromosomal imbalance (ROY, unpublished). The only exception was a cross between *B. juncea* and *B. napus*, designated 75N313, from which it was possible for the first time to transfer JR-gene(s) to a *B. napus* variety (designated $Onap^{JR}$) which was originally susceptible to the disease. It appeared that this type of gene transfer was very rare, perhaps due to the location of JR-gene(s) in the B-genome, common to *B. juncea* (AB) and *B. carinata* (BC) but absent in *B. napus* (AC) (ROY, 1978a). Perhaps this could explain why most of the cultivars from *B. juncea* and *B. carinata* show complete resistance (seedling + adult resistance) whereas *B. napus* shows partial resistance (polygenic). Transfer of JR-gene(s) from B-genome to A- or C-genome through allosyndetic chromosomal pairing depending on the genomic interrelationships could not be ruled out. In fact, the chances for such allosyndetic pairing could increase when chromosomes of alien A, B or C genomes are brought together *de novo* through interspecific crosses (RAJAN & HARDAS, 1964).

From $Onap^{JR}$, through selection in a disease nursery over successive generations (F_4–F_7), it was possible to develop a large number of resistant *B. napus* lines with improved growth, winter vigour and adaptability to cold wet situations. During the same period most lines derived from conventional *B. napus* crosses among the best resistant parents with adult resistance (NR), were gradually lost due to high seedling mortality under severe disease pressure (Tables 1 and 3). Most of the advanced generation resistant selections (F_6–F_7) from $Onap^{JR}$ were found to be still segregating for JR-type complete resistance and are therefore rather unstable. The presence in resistant plants of a complete or centric part of a *juncea* chromosome as a heterozygous translocation is probable, although, cytological study carried out (CHIANG, unpublished) revealed the presence of normal chromosome number ($2n = 38$) of *B. napus* and regular pairing (19 bivalents) in diakinesis. Further study is needed to determine the mode of transfer of JR-gene(s) into $Onap^{JR}$. Results of yield tests (Table 2) indicate normal seed fertility and the high yield potential in some of the $Onap^{JR}$ selections. Some $Onap^{JR}$ lines appeared to be earlier in maturing, a character likely introduced from the Indian *B. juncea* parent.

Besides generalisation, no attempt was made to work out the definite segregation ratio for blackleg resistance based on surviving population. Such estimates are likely to be biased by not including the unaccountable seedling loss at germination, emergence and early growth caused by blackleg or through other diseases like damping off.

With most crop species it is usually impossible to transfer polygenically-controlled disease resistance by interspecific crosses (RUSSEL, 1978). That JR behaved like major gene(s) was supported by our observations of the F_2–F_3 progenies of $Onap^{JR}$ crosses.

This perhaps would explain why some of the interspecific crosses showed dominance for complete resistance at the F_1 level (ROY, unpublished). In the present case, subsequent transfer of JR-resistance gene(s) for Onap^{JR} into other *B. napus* lines through crosses presented no problem even at early segregating generations (F_2–F_3), indicating simple inheritance.

The value of JR-type resistance will be judged by the durability of such resistance, which in turn will be dependent on how such resistance gene(s) could be transferred into a background of minor or modifying genes of resistance (partial resistance) of *B. napus* – and possibly *B. juncea* – origin.

The present study with a large number of F_2–F_3 crosses between Onap^{JR} × *B. napus* (NR) lines indicated that it should be possible to transfer JR-gene(s) into a *B. napus*-type partial resistance or field resistance background. This would not only increase the scope of selection for higher and longer-lasting resistance (VAN DER PLANK, 1971; HAYES, 1973; NELSON, 1978) but also for increased adaptability to cold wet environments (Table 3), yield and oil quality.

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REFERENCES

CARGEEG, L. A. & N. THURLING, 1979. Seedling and adult plant resistance to blackleg (*Leptosphaeria maculans* (DESM.) CES et DE NOT.) in spring rape (*Brassica napus* L.) Aust. J. Agric. Res. 30: 37–46.

HAYES, J. D., 1973. Prospects for controlling cereal disease by breeding for increased levels of resistance. Ann. Appl. Biol. 75: 140–144.

MCGEE, D. C. & G. A. PETRIE, 1979. Seasonal patterns of ascospore discharge by *Leptosphaeria maculans* in relation to blackleg of oilseed rape. Phytopathology 69: 586–589.

NELSON, R. R., 1978. Genetics of horizontal resistance to plant diseases. Ann. Rev. Phytopathol. 16: 359–360.

RAJAN, S. W. & M. W. HARDAS, 1964. Autosyndetic and allosyndetic pairing in *Brassicae*. Ind. J. Genet. and Pl. Breeding 24: 15–21.

ROY, N. N. & J. REEVES, 1975. Breeding better rape and linseed for Western Australia. J. Agric. Western Australia. 16: 95–97.

ROY, N. N., 1978a. A study on disease variation in the population of an interspecific cross of *Brassica juncea* L. × *B. napus*, L. Euphytica 27: 145–149.

ROY, N. N., 1978b. Wesreo – a blackleg resistant rapeseed. J. Agric. Western Australia. 19: 42.

ROY, N. N., 1980. Species crossability and early generation plant fertility in interspecific crosses of *Brassica*. SABRAO J. 12: 43–54.

ROY, N. N. & M. L. POOLE, 1981. Wesroona and Wesbell – high rainfall rapeseed varieties. Western Australian Department of Agriculture – Farmnote No. 109/81.

ROY, N. N., 1983. Transfer of *Brassica juncea* type complete resistance to blackleg into *Brassica napus*. Proc. Australian Plant Breeding Conf. Adelaide, p. 239–240.

RUSSEL, G. E., 1978. Plant breeding for pest and disease resistance. Butterworth and Co. Ltd.

VAN DER PLANK, J. E., 1971. Proc. Seminar on Horizontal resistance to the blast disease of rice. CIAT, Cali-Columbia. 21–26.