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Evidence for a Race-specific Resistance Factor in some Lettuce (*Lactuca sativa* L.) Cultivars Previously Considered to be Universally Susceptible to *Bremia lactucae* Regel

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Summary. Previously undetected race-specific resistance to *Bremia lactucae* (downy mildew) was located in many lettuce cultivars hitherto considered to be universally susceptible to this disease. This resistance factor(s) may also be widely distributed in other cultivars known to carry combinations of already recognised factors R1 to R11. Specific virulence to match this resistance is almost invariably present in pathogen collections. This situation may be either a relic of the evolutionary history of the *B. lactucae* – *L. sativa* asssociation or may reflect a rare mutation in *B. lactucae* for avirulence on all but a few specialised *L. sativa* genotypes.

Key words: *Bremia lactucae* – Lettuce – Race-specific resistance – Downy mildew – Host/pathogen genetics

Introduction

The production of cultivars with resistance to downy mildew (*Bremia lactucae* Regel) has been an objective of lettuce breeders for more than 50 years. As a result, there now exist more than 200 cultivars with some resistance to the disease. However, all the sources of resistance deployed have proved to be race-specific and none has provided durable control of the disease. The genetic interaction between cultivars of *Lactuca sativa* L. and races of *B. lactucae* has been elucidated and eleven resistance factors (R-factors), inherited as major dominant genes, are thought to be matched in a gene-for-gene type of relationship (Person 1959) by eleven factors for specific virulence (v-factors) in the pathogen (Crute and Johnson 1976a; Johnson et al. 1977, 1978). Lettuce cultivars can now be classified according to the different combinations of R-factors they carry (Crute and Johnson 1976a, b; Johnson 1978; Crute 1979a, b). At present, probably 25 of the possible 2048 (2^{11}) different combinations of R-factors exist in lettuce cultivars. Some combinations (e.g. R3 and 7) may be represented by more than 40 cultivars while others (e.g. R5) are found in only a single cultivar. It is sometimes impossible to demonstrate the presence or absence of certain R-factors (e.g. R1, Crute and Johnson 1976a) in a cultivar when appropriate diagnostic isolates of *B. lactucae* are not available. For this reason, postulated R-factor complements of cultivars require amending from time to time as new information becomes available. The virulence phenotype of pathogen isolates may now be defined in terms of the v-factors they carry as determined by inoculation onto appropriate indicator cultivars.

Implicit in studies of the genetics of host/parasite interactions, is the concept that some host genotypes are 'universally susceptible' i.e. they carry no race-specific resistance and will prove compatible with all isolates of the pathogen regardless of virulence phenotype. In the case of the *B. lactucae*/*L. sativa* system, several different cultivars have been employed by different researchers in this role, with cv. 'Hilde' being most frequently used. It has been assumed that the lettuce cultivars not amongst those mentioned in the literature as showing resistance to some races of *B. lactucae* are 'universally susceptible'. However, observations during studies on variation for specific virulence in the Czechoslovakian *B. lactucae* population suggested that this assumption may be incorrect. Tests were, therefore, carried out on a range of genotypes and evidence is presented in this paper which demonstrates that many lettuce cultivars previously considered to be universally susceptible do carry one or possibly two hitherto unrecognised R-factors which are only rarely unmatched by v-factors in the pathogen. This finding is of little practical significance for future lettuce breeding but has some interesting theoretical implications.

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Materials and Methods

Seed of 33 lettuce cultivars considered to carry no race-specific resistance to *B. lactucae* were obtained from commercial seed companies. The cultivars were primarily types used for outdoor cultivation and included butterhead, cos and crisp types. Seedlings were raised under standard soil-free conditions in a growth room using previously described methods (Crute and Johnson 1976a; Lebeda 1979a, b). Inoculations were made 7-10 days after sowing when cotyledons were fully expanded. Seedlings were dipped in a suspension of 1-2 day-old *B. lactucae* spores which had been washed by low speed centrifugation and were then incubated under illumination at approximately 15°C. Assessments of sporulation intensity were made on at least three occasions from 6-14 days after inoculation and sporulation on each seedling was graded on a 0-3 scale (Crute and Dickinson 1976). In each test, three replicate batches of each cultivar were inoculated and a minimum of twenty seedlings per cultivar were used for each test although there were frequently more. Results were expressed as a percentage of the maximum possible score for each batch of seedlings, e.g. ten seedlings scoring 3 and ten scoring 2 out of a batch of twenty gives an intensity of 83.3%.

A compatible (susceptible) reaction type was characterised by copious sporulation, usually on all seedlings, commencing after 5-6 days and reaching a final intensity of close to 100% by day 14 or earlier. In contrast, an incompatible (resistant) reaction was distinguished either by the complete absence of sporulation or by the occurrence of sparse sporulation, delayed in its time of appearance and associated with necrotic flecking and/or chlorosis. When sporulation did occur in such cases, the intensity at the final assessment did not exceed 25%. In all tests, the integrity of the isolates used was confirmed by inoculating appropriate differential cultivars at the same time. Three isolates were used at the National Vegetable Research Station, Wellesbourne, Warwick (England) which between them were considered to demonstrate the presence or absence of R1 to R11 in unknown lettuce genotypes. The virulence phenotypes were as follows: isolate W5, v 1, 2, 4; isolate IM43, v 5, 6, 7, 8, 10; and isolate Tv (referred to previously as 74/T), v 1, 2, 3, 4, 5, 6, 7, 8, 9, 10. It can be ssen that susceptibility to all three of the above isolates indicates that none of R1 to R11 were present in the cultivar under test. At the Plant Breeding Station, Smrzice (Czechoslovakia), the isolate CS6 with virulence phenotype v 2, 4, 5, 10 was primarily used. Initially this isolate had been recorded by Lebeda (1979c) as being compatible with cv. 'Hilde', but it is now believed that this record resulted from a misinterpretation of an incomplete resistance response (Crute and Norwood 1978). Further studies indicated that CS6 was avirulent on cv. 'Hilde' and led to the present investigation. Other isolates from the series CS1 to CS12 were also used as required (Lebeda 1979c, 1980a, b; Lebeda et al. 1980 for details).

Results and Discussion

On the basis of their reactions to isolates W5, IM43 and Tv (Table 1) it can be concluded that for all but three of the cultivars tested ('Imperial Winter Reselected', 'Winter Imperial' and 'Asmer Spring Market') the original assumption that they did not carry R1 to 11 was correct. 'Asmer Spring Market' was subsequently found to be synonymous with cv. 'Blondine' which is known to carry R1, and further tests with the CS series of isolates confirmed the presence of R1 in these three cultivars (resistant to all isolates lacking v1). Despite the fact that R1 to 11 were not located in the remainder of cultivars, all but seven proved to be resistant to isolate CS6. The following seven cultivars may still be considered to be 'universally susceptible': 'Darka', 'Cobham Green', 'Lobjoits Cos', 'Imperial Winter', 'Virilde', 'Valdor' and 'Winter Day'. The remaining 24 cultivars must be assumed to carry one or more previously unrecognised R-factors contributing resistance to CS6 but matched by the necessary v-factors in all other isolates tested. In certain cultivars (e.g. 'Hilde' and 'Suzan') the new R-factor was incomplete in its expression (Crute

Table 1. Reaction of 33 lettuce cultivars, previously considered to be universally susceptible, to four isolates of *Bremia lactucae*

	% Sporulation intensity			
Isolate	W5	IM43	Tv	CS6
Virulence phenotype	1,2,4	5,6,7,8,10	1-10	2,4,5,10
Cultivar				
Imperial Winter Reselected	100	0	93 (6)	0
Winter Imperial	100	0	100	2^{a}(2)
Asmer Spring Market	100	1^{a}(2)	97 (6)	0
Darka	100	100	100	64 (4)
Cobham Green	100	99 (2)	99 (2)	77 (14)
Lobjoits Cos	100	100	100	97 (6)
Imperial Winter	100	100	–	97 (5)
Virilde	100	83 (19)	100	99 (2)
Valdor	100	96 (3)	100	81 (11)
Winter Day	67 (0)	97 (6)	88 (6)	71 (10)
All the Year Round	64 (22)	83 (7)	100	2^{a}(4)
Arctic King	100	98 (3)	100	3^{a}(4)
Aurelia	71 (4)	98 (3)	98 (3)	1^{a}(1)
Buttercrunch	100	100	98 (4)	1^{a}(2)
Constant Heart	100	100	–	5^{a}(6)
Continuity	100	86 (20)	98 (3)	0
Empire	100	100	100	0
Favourite	100	96 (3)	100	0
Hilde	100	100	100	11^{a}(8)
Ilo	100	–	100	6^{a}(6)
Irma	100	100	90 (9)	0
Ithaca	78 (15)	100	60 (16)	0
Massa	100	100	100	3^{a}(4)
New Market	100	100	–	6^{a}(2)
New York	100	98 (4)	97 (6)	0
Novir	100	100	90 (5)	0
Pennlake	100	100	97 (3)	0
Plena	100	88 (16)	82 (13)	14^{a}(7)
Prado	100	100	100	3^{a}(6)
Suzan	100	100	100	21^{a}(18)
Unrivalled	–	–	–	0
Webbs Wonderful	100	100	87 (23)	0
Windermere	100	100	100	0

Figures in parentheses are standard deviations of three replicates

[a] Incomplete resistance reaction

and Norwood 1978) allowing sparse sporulation associated with macroscopically visible tissue necrosis (hypersensitivity).

At least a further nineteen cultivars (not listed in Table 1), i.e. 'Unrivalled', 'Melnický Máj', 'Tenax', 'Magna', 'Amerika', 'Rigoletto', 'Neckarriessen', 'Attractie', 'Kraganer Sommer', 'Viruzan', 'Boheme Blonde Grosse', 'Neusidler', 'Wonder von Stuttgart', 'D'Hivero Verieres', 'Dry Weather', 'Grosso', 'Relly', 'Maya and Steran', thought not to carry factors R1 to R11, but which were not tested with isolates W5, IM43 and Tv in this study, were also solely resistant to CS6. They probably therefore also carry the new R-factor.

Within the group of cultivars identified as resistant to CS6, six ('Empire', 'New York', 'Webbs Wonderful', 'Windermere', 'Pennlake' and 'Continuity') appeared to carry a further or different unrecognised factor for race-specific resistance. These cultivars in addition to being resistant to CS6, also proved resistant to isolates CS1, CS4, CS5, CS7, CS10 and CS12. This reaction pattern in the proven absence of R1 to R11 could only be explained by the presence of one or more further R-factors. With the exception of cv. 'Continuity', this group of cultivars are all of the crisp type, probably with common ancestry.

Since CS6 is the only isolate known to be capable of detecting this previously unrecognised R-factor, and because of its comparatively simple virulence phenotype, it is only possible to determine the presence or absence of the new R-factor in cultivars lacking factors R1, R3, R6, R7, R8, R9 and R11. For this reason, it can at present only be assumed *not* to be present in the following cultivars with known resistance genotypes: 'Sucrine' (R10), 'Tornado' (R10 or R5), 'Valmaine' (R5), 'May King', 'Amplus', 'Deciso', 'Deci-Minor', 'Knap', 'Miranda', 'Vitesse', 'Triumf', 'Seaqueen', 'Kral Majé', 'Videnskу' and 'Amanda-plus' (all with R2 and R4), together with the seven cultivars listed above.

It would be interesting to determine how many cultivars carrying the R2 and R4 combination also carry the new R-factor since in only one of these (cv. 'Noran') has it been located. It would also be interesting to ascertain if primitive *L. sativa* genotypes occurring as 'land-races' or semi-wild populations in certain regions also carried this character. Lebeda (1981) reported that the Czechoslovakian host population from which his study of the *B. lactucae* population was made consisted primarily (46%) of cultivars carrying R2 and R4. A smaller proportion of the host population (4%) was of R0 types (i.e. formerly considered to be universally susceptible but probably carrying the new R-factor). If this previously undetected R-factor is not present in most types carrying R2 and R4 then, in contrast to other areas in Europe, there may not have been such intense selection for virulence to match it in Czechoslovakia. However, avirulence on cv. 'Hilde' was found only once out of 300 isolates studied in Czechoslovakia.

It is only possible to speculate on reasons for the apparent frequent occurrence of the new R-factor in lettuce cultivars together with the almost invariable occurrence of virulence to match it in samples from the *B. lactucae* population. Van der Plank (1975, p. 163) envisaged a situation where a specific virulence factor may occur almost universally when he wrote: 'There could conceivably be virulence that, even in the absence of matching resistance in the host, exists so abundantly as to be almost universal. If so, we are unlikely to know of it. It needs avirulence to identify a resistance gene, and a resistance gene to identify virulence; and the occasional avirulence that arose by mutation would be difficult to capture'. Van der Plank (1975, 1978) suggested that loci determining specific virulence in a pathogen may be 'even more numerous than we are aware' and considered that pathogen alleles at virulence loci and host alleles at resistance loci probably had other primary functions distinct from the determination of specificity. Isolate CS6 therefore may be interpreted as carrying a rare and unfit mutation to avirulence. The exact frequency with which the new R-factor occurs within the host population cannot be determined because it is not possible to demonstrate its presence or absence in certain modern cultivars. However, because the new R-factor is frequently encountered by the *B. lactucae* population it cannot be assumed that it simply corresponds to a 'weak' gene (sensu Van der Plank 1968). By contrast, several other genes for race specific resistance to *B. lactucae* in lettuce (e.g. R1, R5, R9 and R10) can be considered as 'weak' by Van der Plank's (1968) terminology since they occur in a few non-commercial cultivars yet virulence to match them occurs frequently (Wellving and Crute 1978; Crute et al. unpublished; Lebeda 1981).

This previously undetected R-factor may have contributed effective resistance at some time in the evolutionary history of the association between *Lactuca* and *B. lactucae* and for this reason may have become frequent and widely distributed followed by a parallel increase in the frequency of the matching virulence. In this sense, the results reported here may have identified a relic of the evolution of the host-pathogen association and of gene matching for compatibility. If this were the case it would represent an example of transient polymorphism (Leonard and Czochor 1980). Alternatively, this R-factor may have occurred in the progenitor from which cultivated forms of *L. sativa* developed by chance. Such a factor could then only have been eliminated from lettuce genotypes as a result of mutation or during the relatively recent process of artificial hybridisation for breeding purposes. It is worth recording that this new R-factor has been located primarily in outdoor lettuce types (day-neutral types). Almost all types for protected cropping (long-day types) have their origins in a narrow genetic base (cvs 'Meikoningin' and 'Gotte à Forcer'). Interestingly, CS6 is compatible with 'May King' (synonymous with 'Meikoningin' and carrying R2 and R4) which may indicate a different origin for these two physiologically distinct groups of lettuce.

Van der Plank (1975, 1978) suggests that the loci for resistance in the host and virulence in the pathogen primarily control functions other than the determination of specificity which he is considered to be secondary and for which a mechanism is proposed. Within this interpretation, the previously undetected R-factor may be of importance to *L. sativa* in its primary role; most lettuce genotypes probably carry it. As a consequence, the *B. lactucae* population had to evolve to be compatible. Mutations at a locus in the pathogen which dismantle this compatibility would be seen as avirulence and would be lethal if it were not for the occasional occurrence of lettuce genotypes lacking the character. If the mutant can be isolated, the host gene is identified as an R-factor. Presumably, rare but 'strong' R-factors (sensu Van der Plank 1968) are less satisfactory or important constituents of the host genome in their primary role and mutation towards compatibility in the pathogen also occurs at the expense of the efficiency of the primary function in the pathogen.

The process of evolution in specialised plant pathogens such as downy mildews appears to be in the direction of greater specificity. For example, the species *B. lactucae* will attack 36 different genera of Compositae but individual collections are specialised such that they are compatible only with genotypes of a restricted number of host species within a single genus (Crute and Davis 1977). These data may reflect a further process of specialisation towards only certain genotypes within a single species (*L. sativa*) although, for practical reasons, it has been more common to discuss variation for specific virulence to host genotypes in the context of changes towards increasing host cultivar range (increased flexibility) and hence the erosion of previously effective resistance. In this case the flexibility of the pathogen isolate CS6 is reduced by comparison with the bulk of the population and it would be interesting to determine experimentally if there had been a commensurate increase in fitness on the host genotypes with which the isolate is compatible.

The use of cv. 'Hilde' as a common 'universally susceptible' cultivar may have hindered the prior recognition of this new R-factor since potentially heterogenous field collections of *B. lactucae* would frequently be multiplied on this cultivar prior to virulence testing resulting in the elimination of all components of the population avirulent to it. Alternatively, if mass spore isolates from a field crop were used directly to inoculate a set of differential genotypes, a susceptible reaction on cv. 'Hilde' would still be recorded even if avirulent mutants (see above) were present at low frequencies within the spore population. In this context, Crute and Dickinson (1976) recorded a low frequency of apparently incompatible penetrations following inoculation of a range of cultivars thought to be 'universally susceptible'. Isolate CS6 was originally isolated from a single lesion on cv. Triumf (R2 + R4) and has been maintained since on cv. 'Kamýk' (R4).

Since virulence to match the newly identified R-factor occurs so frequently and is widely distributed, it is suggested that it is unnecessary to make *B. lactucae* virulence phenotype descriptions more cumbersome by recording its almost universal presence (this follows the arguments of Van der Plank (1968 p. 77) in discussing virulence to match 'weak' R-genes to *Phytophthora infestans* DeBary in potatoes). It is also suggested, because of this situation, that the new R-factor is not specifically numbered, possibly until inheritance studies are conducted. A preliminary study of F_2 progeny from crosses between cvs 'Hilde' or 'Borough Wonder' (both carrying the new R-factor) and cvs 'May King', 'Valmaine and Sucrine' (all susceptible to CS6) inoculated with CS6 gave inconsistent data which were uninterpretable in terms of simple Mendelian ratios.

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