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OPPORTUNITIES FOR BIOTECHNOLOGY RESEARCH ON SORGHUM STALK ROT AND VIRUS DISEASES

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ABSTRACT

Although considerable progress has been made using conventional technology in identification and characterization of viral and stalk rot diseases of sorghum, there is still an incomplete understanding of the genetics of resistance, and many viral diseases remain uncharacterized. Biotechnology (cDNA) will play an increased role in virus detection and identification, and in marker assisted selection in breeding for sorghum virus resistance, and to discern the genetic basis of resistance to the stalk rot complex quickly and more precisely. Biotechnology will also aid in the identification of quantitative trait loci (QTL) associated with specific *Fusarium* and *Macrophomina* stalk rots responses and drought tolerance.

***Key Words*: cDNA, conventional breeding, drought tolerance, marker assisted selection, virus resistance**

RÉSUMÉ

Bien que des progrès considérables aient été réalisés avec la technique conventionelle d'identification et de caractérization des maladies virales et de stalk rot de sorgho, il reste à comprendre la résistance génétique et à caracteriser beaucoup de maladies virales. La biotechnologie (DNA) jouera un role prépodérant dans la détection et l'identification des viroses, dans l'amélioration de la résistance aux virus à l'aide de la séléction, grâce aux marqueurs et dans la détermination rapide et précise des bases génétiques de résistance au complèxe stalk rot. La biotechnologie aidera aussi à l'identification de traits quantitatifs (QLT) au loci associés au fusarium et au Macrophomia stalk rots et à la tolérance à la secheresse.

***Mots Clés:* DNA, amélioration conventionnelle, tolerance à la sécheresse, séléction avec marqueurs, résistance à la virose**

VIRUS DISEASES

Sorghum viruses are distributed worldwide and cause economic damage to the host. The first virus disease described in sorghum was sugarcane mosaic, identified by Brandes and Klaphaak (1923). Since then 23 viruses have been identified within 12 groups; and in addition, other viruses or isolates of potyvirus remain unclassified (Table 1). Seven viruses have been experimentally transmitted to *Sorghum* spp. (Table 1). According to Peterschmitt *et al.* (1991), it appears that MStV-sorg causes the most important virus disease of sorghum in the Indian subcontinent, with an incidence of 10%. However, the potyvirus group are the most widespread and prevalent viral pathogens of sorghum (Giorda, 1993).

Although many viruses related to sorghum have been isolated and characterized, several still have yet to be identified. Antisera to some of them are

TABLE 1. Viruses naturally and experimentally transmitted to sorghum

Virus group	Transmission
BROMOVIRUS*	
Brome mosaic virus, BMV	Nematode, beetle
CUCUMOVIRUS	
Cucumber mosaic virus, CMV (Exp.)	Aphid (NP)
FUROVIRUS*	
Sorghum chlorotic spot virus, SChSV	Fungus
Peanut clump virus, PCV	*Polymixa graminis*
GEMINIVIRUS*	
Maize streak virus, MSV (Exp.)	(*Ciccadulina* spp.) (LH)
LUTEOVIRUS	
Barley yellow dwarf virus, BYDV	Aphid (persistent)
MCDV	
Maize chlorotic dwarf virus, MCDV	*Graminella nigrifrons*, LH
MCMV	
Maize chlorotic mottle virus, MCMV (Exp.)	beetles
POTYVIRUS	Aphid (non-persistent)
Johnsongrass mosaic virus, JGMV	
Maize dwarf mosaic virus, MDMV	
Sugarcane mosaic virus, SCMV	
Sorghum mosaic virus, SrMV	
Unclassified[1]	
MDMV-China	
SOMV-India	
MDMV-Venezuela	
MDMV-D	
MDMV-Honduras	
SCMV-Honduras	
MDMV-Africa	
SCMV-Africa	
Others	
REOVIRUS*	
Sugarcane Fiji Disease	*Perkinsiella* spp.(LH)
Maize rough dwarf virus, MRDV	*Delfacodes* spp.(PH)
RHABDOVIRUS	
Sorghum stunt mosaic virus, SSMV	*Graminella* spp. (PH)
Maize mosaic virus, MMV	*Peregrinus* spp. (PH)
SOBEMOVIRUS	
Panicum mosaic virus, PMV (Exp.)	
TENUIVIRUS*	
Maize stripe virus, MStpV	
Maize stripe virus, sorghum-isolate, MStV-Sorg	*Peregrinus* spp.
Sorghum red stripe virus, SRSV	*Peregrinus* spp.
Rice stripe virus, RSV (Exp.)	leafhopper
(?) Iranian wheat stripe virus, IWhSV (Exp.)	*Unkanodes tanasijevici*
Maize yellow stripe virus, MYSV (Exp.)	(Delphasid) leafhopper
Others[2]	*(Cicadulina* spp.)
Sorghum yellow banding virus, SYBV	
Sugarcane chlorotic mottle virus, SCMV	
Johnsongrass chlorotic stripe mosaic virus, JCSMV	
Sorghum mosaic disease, SMd	

*Not mechanically transmitted
[1]Unclassified within the potyvirus class or subgroups.
[2]Unclassified within the subgroups.

not readily available and others remain uncharacterized at the gene sequence level (Table 1). Moreover, the genetic basis of resistance to virus diseases is incompletely understood. Thus, several steps should be considered sequentially or simultaneously, depending on the host-pathogen system, the geographical area and the status of the technology to assist in resolving these issues: (i) biotechnological methods to assist virus detection and identification, (ii) marker-assisted selection in breeding for sorghum virus resistance, (iii) sorghum virus-engineered resistance, and (iv) a cost/benefit analysis for the application of biotechnological strategies to assist in sorghum improvement.

Sorghum virus detection and identification. Serological methods, using polyclonal antisera, have been widely applied for many years for the detection and identification of plant viruses. A limitation in the use of polyclonal antisera is the inability to readily discriminate between strains of a virus. One example is the potyvirus group.

Numerous strains which differ mainly in biological properties, such as host range and/or pathogenicity, have been reported on sorghum (Giorda, 1988). Attempts to identify and classify distinct sorghum potyvirus and their strains have frequently been hampered by the presence of variable proportions of cross-reacting antibodies in the antisera. This limitation has been overcome by the development of monoclonal antibodies and other serological approaches such as virus-specific antibodies using affinity chromatography (Shukla *et al.*, 1988; Ward and Shukla, 1991). A number of other techniques can be used for sorghum virus detection including ELISA and radioactive and nonradioactive nucleic acid probes (Chu *et al.*, 1989; Dietzgen *et al.*, 1994). However, some of these techniques are expensive and labour-intensive.

Other biotechnological approaches such as cDNA probes have facilitated virus detection (Hamilton, 1992). Nucleic acid hybridization is a powerful technique for detection of complementary nucleic acid sequences and is being increasingly used for diagnosis of the potyvirus and other virus groups.

Henson and French (1993) reviewed the application of the polymerase chain reaction (PCR) to pathogen diagnosis. PCR permits the amplification of specific DNA sequences and offers several advantages compared to more traditional methods of diagnosis (Bariana *et al.*, 1994). The reverse transcription polymerase chain reaction (RT-PCR) assay simultaneously tests a sample in one tube for the presence of different viruses (Bariana *et al.*,1994; Smith and van de Velde, 1994). This new technique provides a low cost tool for research into the incidence, ecology and epidemiology of sorghum virus diseases.

RFLP-assisted breeding for sorghum virus resistance. There are various ways in which damage from plant viruses can be avoided. Each control method can be effective alone or when combined with others. However, the most economical, biologically safe and practical approach to control sorghum viral diseases is the planting of resistant or tolerant cultivars.

Genetics of resistance to sorghum virus diseases have been described for the potyvirus group (Conde *et al.*, 1976; Persley *et al.*, 1977; Giorda, 1988). Considerable variation among isolates of the subgroups MDMV, SCMV, JGMV and SrMV have been demonstrated (Toler *et al.*, 1982; Tosic *et al.*, 1990; McKern *et al.*, 1991).

In Australia, the inheritance of mosaic and necrotic reaction was studied by Persley *et al.* (1977). Two independent gene loci are involved in the control of the red leaf (*rlf*) and red stripe (N) reactions in sorghum. Presence of the homozygous recessive allele *rlf* conditions the expression of the necrotic red leaf reaction following exposure of plants to low temperature.

Plants express only mosaic symptoms in the presence of the dominant allele *RLF* and the absence of the *N* gene. The presence of a single dominant host gene *N* results in the expression of necrotic red stripe symptoms. Conde *et al.*(1976) described immunity conditioned by the *Krish* gene. *Krish* resistance depends on a single gene *K* with resistance (symptomless plants) dominant over susceptibility. The relationship of the *Krish* gene with the *N* gene which controls the mosaic (*kk nn Rlf-*), red leaf (*kk nn rlfrlf*) and the red stripe (*kk N- rlfrlf*) reactions, has not been definitely established. It could be either closely linked or act as an additional allele at the *N* locus (Persley *et al.*, 1977; Giorda, 1988). The resistance in *Krish*

is monogenic, dominant and has been extensively used since 1971 in breeding programmes worldwide to develop cultivars resistant to different strains of JGMV, SCMV, SrMV and MDMV. In 1985, red stripe symptoms occurred in a hybrid with *Krish* resistance from one parent in Australia (Persley *et al.*, 1987). The appearance of isolates able to overcome *Krish* resistance puts into questions the durability of their monogenic resistance for potyvirus control.

Studies on the genetics of resistance to the sorghum potyvirus group have attempted to fit the progeny of resistant x susceptible crosses into symptom response classes. The genetics of resistance to MDMV-A was based on disease severity as measured by area under a disease progress curve (AUDPC) and virus accumulation measured as ELISA values. The diallel analysis for virus accumulation showed that the genetic variation is mainly additive, and dominance for resistance to virus accumulation accounted for the non-additive variation (Giorda, 1988).

Field screening in areas of natural disease occurrence and large-scale artificial inoculation procedures have contributed to the improved MDMV/SCMV tolerance of commercial sorghum cultivars.

A high density genetic linkage map is available for sorghum (Pereira *et al.*, 1994; Xu *et al.*, 1994). Thus, RFLPs could be used to assist in breeding and selection for MDMV-A or SCMV-Jg (JGMV) resistance even though it is monogenic dominant, and thus selection is already relatively inexpensive and straight forward. Should we use RFLPs to assist in the selection for this type of trait with high heritability, that is easy to evaluate and to improve through conventional means? Although it is still far from "low tech", more effective selection and fewer generations would be required in breeding using RFLPs. It is valuable when a trait is recessive (example, resistance to MDMV-B or MDMV-V), difficult to assay (viruses not mechanically transmitted, such as MStV-Sorg), and in backcross breeding programmes to select simultaneously for the desired chromosomal regions (for instance, genes controlling disease resistance) and against unwanted chromosomal segments, such as those that might be introduced while backcrossing from exotic germplasm or low yielding parents with poor agronomic characteristics. This is very important since it is possible to access genes from wild relatives or photosensitive varieties where most sources of resistance are found. Marker-assisted selection should be done to pyramid genes into a single cultivar resistant to different viruses or strains of the same pathogen.

The genetic basis of sorghum virus resistance as well as screening for resistance is often confusing because natural infection by different viruses or strains causes similar or distinctive symptoms. Utilization of RFLPs would speed up identification or detection of sources of resistance and it could also be used to describe different types of gene action.

Pratt *et al.* (1992) discussed the rationale involved in deciding whether or not to use RFLPs to assist in the improvement of yield and host plant resistance to maize viral pathogens. They stated that the more difficult the traits are to select by conventional methods, the stronger the argument to use RFLP marker-assisted breeding. "Traits that can be easily evaluated with conventional approaches are probably more cost effective". Similar arguments might be applied to sorghum improvement for virus resistance. Specific application of RFLPs will depend on research priorities defined for each region based on the constraints, economic development, consumer and producer demand (food, industrial, or feed) and defined market niches and policies.

Sorghum virus-engineered resistance. Coat protein-mediated (CP) resistance has been successfully demonstrated for viruses belonging to 10 groups including potyvirus (Beachy *et al.*, 1990; Beachy, 1993). This is the most promising strategy being used in research on virus resistance and is based on the phenomenon of cross-protection. The protection acts only against related viruses showing a high level of homology in the amino acid sequence of their coat protein (Beachy *et al.*, 1990).

CP-mediated resistance segregates as a dominant trait. Genetic stability of this resistance is expected to be the same as for any other gene from conventional breeding. For instance, a single point mutation of a vital amino acid might affect the level of resistance similarly to any other monogenic resistance gene (Stiekema *et al.*, 1993).

This argument disagrees with that of Zoeten (1991), who indicated that the utilization of CP-mediated resistance to control virus diseases could lead to the development of new virus strains. The CP-mediated resistance is of a multiple type: resistance to inoculation, to symptom expression, to virus spread within the plant and to virus multiplication. Stiekema *et al.* (1993), speculating about durability of CP-mediated resistance, indicated: "If the presence of viral coat protein in each case blocks viral infection at different levels e.g. the level of viral RNA dismantling, the level of viral RNA replication and the level of systemic spread of the virus in the plant, it is most likely that the resistance will prove to be durable. In that case the virus has to mutate in such a way that none of the three vital processes will be inhibited by the presence of the coat protein. The durability will be determined by the product of the probabilities of circumvention by virus mutation of each of the individual infection blocks".

Recently, Murry *et al.*(1993) developed transgenic maize plants expressing MDMV-B CP. The more striking results are that regenerated plants were resistant to MDMV-A, MDMV-B and to mixed inoculations of MDMV and MCMV. This progress encourages future research on sorghum virus diseases to provide alternatives for strengthening or supplementing existing genetic resistance. Since CP-mediated resistance protects from related viruses, we can speculate on the possibility of introducing MDMV-A CP into a high yielding female sorghum line (A/B) without introducing undesirable traits, and provide resistance to other virus or strains of the potyvirus group infecting sorghum that share about 60% homology in their amino acid sequence.

If the monogenic highly effective *Krish* gene lasted almost 15 years in Australia and remains effective elsewhere, the question arises on how long will CP-mediated resistance will last in transgenic sorghum plants?

Specific applications of the different biotechnologies for control of sorghum virus diseases will depend on the local problem, research facilities, and training capacities of individual investigators. For instance, in Argentina, we have characterized and identified MDMV-A. It is the most important and prevalent virus in this sorghum growing region. Sources of resistance have been detected and incorporated in the parental lines. The use of RFLPs will enhance our breeding programme by permitting the development of high yielding females with good general combining ability and resistance to different diseases, such as sorghum downy mildew, MDMV-A, and pests, such as greenbug, by pyramiding the resistance genes from different B or R line sources. However, sorghum is declining in importance in Argentina as well as other Latin American countries. Changes in production and consumption have led to pessimism about the future impact of new technologies, and leading to shift of potential investment in sorghum research to cash crops such as soybean, maize, wheat, potato and cotton.

In other regions, such as different areas of Africa and Central or South America, detection and identification of sorghum viruses is a necessary step. Genetics of resistance to several important virus diseases is lacking or incomplete because they are difficult to work with under field conditions. In these situations, biotechnology coupled with traditional methods may greatly facilitate studies leading to disease control.

In summary, molecular markers such as RFLPs are likely to have a major impact on developing countries, providing non-radioactive techniques and less expensive probing techniques. "RFLP technology brings together molecular genetics and classical plant breeding"(Young *et al.*, 1992). Its utilization for backcross breeding or for pyramiding different resistance genes and defense genes, for instance, would play an important role in sorghum crop improvement.

SORGHUM STALK ROT DISEASES

Stalk rots are widely distributed sorghum diseases and are induced by several organisms. *Macrophomina phaseolina* (Tass) Goid and *Fusarium moniliforme* Sheld are the most prevalent pathogens causing charcoal rot and Fusarium root and stalk rot, respectively (Pande and Karunakar, 1992; Giorda *et al.*, 1995).

Yield losses from 15 to 60% may occur on susceptible cultivars depending on the season and region (Mughogho and Pande, 1984; Anahosur, 1992; Pande and Karunakar, 1992; Giorda *et al.*, 1995). Stalk rots account for an average loss of 4-

8% of the sorghum crop in USA (Claflin and Leslie, 1993). However, good damage estimates from farmers' fields are lacking and difficult to assess because of the association of the disease with physiological stresses from water and heat or from stress caused by greenbug feeding and different crop management situations. Stalk rot is generally caused by more than one pathogen under natural conditions (Pande and Karunakar, 1992). The complex etiology of charcoal rot and Fusarium stalk rot makes them challenging to work with.

Mihail *et al.* (1988) and Pande and Karunakar (1992) and reviewed the present state of knowledge and progress on different aspects of sorghum root and stalk rot diseases with emphasis on charcoal rot. Fewer studies have been conducted on the etiology, mode of action and host pathogen interactions of Fusarium stalk rot.

Good control of these diseases can be obtained through cultural practices of soil and water management techniques, and by avoiding or decreasing greenbug damage by applying insecticides or growing resistant cultivars (Doupnik, 1984; Evangelista and Tangonan, 1991). However, this approach is difficult to manage, considering that most of the area planted with sorghum is rainfed and drought-prone.

Host resistance is the most practical long-term solution. However, it has been difficult to identify stable sources of resistance to charcoal rot or Fusarium root and stalk rot in cultivars with good agronomic traits and resistance.

Both diseases need predisposing conditions (Pande and Karunakar, 1992; Giorda *et al.*, 1995) which affect the amount of stalk rot and lodging, complicating the selection of resistant genotypes as well as studies on host-pathogen interactions. The source-sink relationship plays an important role in the selection for resistance to lodging and to both stalk rot diseases. Characters that affect this relationship such as grain yield and maturity must be considered when selecting for those traits (Henzell *et al.*, 1984; Rosenow, 1984). The size of the carbohydrate sink of the panicle is important in determining the level of stress necessary to induce stalk rot predisposition.

Different screening techniques, based mainly on senescence or green leaf retention, lodging percentage, and extent of pith degradation, have been used to select for stalk rot resistance. However, the rating was not always done within maturity and grain yield classes. Lack of consistent selection pressure because of differences in the predisposing physiological or biotic stresses, because of differences in maturity or sink size, cause variations in the host response and, consequently, influence the effectiveness of the selection for disease reaction.

Several workers have studied the genetics of resistance to *F. moniliforme* or *M. phaseolina* (Bramel-Cox *et al.*, 1988; Bramel-Cox and Claflin, 1989; Rosenow, 1992; Tenkouano *et al.*, 1993). Selection for resistance to *M. phaseolina* was done indirectly through selection for drought tolerance, using nonsenescence as the main criterion. Rosenow (1984) reported significant correlations between nonsenescence, lodging resistance and charcoal rot resistance in Texas, USA. Similar results were obtained by Mughogho and Pande (1984) for charcoal rot in India. Henzell *et al.* (1984) bred directly for lodging resistance and, thus, indirectly for stalk rot resistance since lodging ("stem collapse") is the ultimate effect of stalk rot in Australia. However, nonsenescence was also identified by this author as the most important plant character related to stalk rot (*M. phaseolina* and *F. moniliforme*), lodging, and postfloral drought resistance. Maunder (1984) also mentioned: "selection for stiff stalk and drought tolerance, especially of the nonsenescent type, combined with high yield will be more productive than breeding for charcoal rot resistance alone".

Fusarium stalk rot resistance has also been indirectly screened for by selecting individual plants or populations with lodging resistance, greenbug resistance or nonsenescence characteristics. Henzell *et al.* (1984) suggested that the reaction of genotypes to *M. phaseolina* and *F. moniliforme* may be inherited independently and that there may be a correlation between lodging resistance and resistance to *M. phaseolina* but not to *F. moniliforme*.

Bramel-Cox *et al.* (1988) reported that the inheritance of resistance to *F. moniliforme* and *M. phaseolina* based on artificial inoculation techniques is controlled by a multiple-locus complex with distinct heterotic patterns. The expression of resistance appeared to be dependent

on the environment (both organisms) or maturity (*M. phaseolina*). Duncan and de Millano (1995) and Rosenow (1992) indicated recessive/ intermediate inheritance patterns for both charcoal rot and Fusarium stalk rot. Recent studies (Tenkouano *et al.*, 1992; 1993) on the genetics of nonsenescence and charcoal rot resistance in sorghum determined that nonsenescence was regulated by dominant and recessive epistatic interactions between two nonsenescence-inducing loci and a third locus with modifying effects. This was also true for charcoal rot resistance. The authors concluded that "nonsenescence alone can not account for, and should not be used as the sole breeding criterion for, resistance to charcoal rot in sorghum".

MIGHT BIOTECHNOLOGY SPEED UP SORGHUM IMPROVEMENT FOR STALK ROTS RESISTANCE?

Fusarium root and stalk rot. Little is known about the host-pathogen interactions, however, progress was made by developing Fusarium stalk rot-tolerant cultivars screened for characters other than resistance to the pathogen *per se*. Three main characters have been mentioned in the literature as associated with tolerance to Fusarium stalk rot: lodging resistance, nonsenescence and greenbug resistance (Henzell *et al.*, 1984; Mughogho and Pande, 1984; Rosenow, 1984; Giorda *et al.*, 1994). The first two might be considered phenotypic expressions of postflowering drought tolerance. Drought tolerance is a complex trait influenced by the interactive effects of many genes. Ejeta (1993) has identified several "quantitative trait loci" (QTL) associated with pre- and post-flowering stress tolerance.

Since complex traits are involved, it might be possible to discern the genetic basis of the resistance quickly and more precisely with RFLPs. Tolerance to moisture stress under post-flowering drought conditions is manifested by a stay-green (nonsenescence) phenotype. Most of the genotypes with enhanced drought tolerance often show limited yield potential.

Several reports have summarized the application of different molecular markers to tag useful genes and to determine the gene action (Paterson *et al.*, 1988; Tanksley *et al.*, 1989; Keim *et al.*, 1990; Ueng *et al.*, 1992; Hulbert, 1995; Jones and Canada, 1994). Based on these observations, efforts should concentrate on identifing QTL loci associated with specific Fusarium stalk rot responses under uniform conditions for disease development. In this way, once traits (post-flowering drought tolerance and Fusarium root and stalk rot resistance genes) are tagged, it might be possible to use RFLPs to improve high yielding susceptible lines by assembling combinations of these desirable genes.

Studies on *Fusarium* spp. taxonomy and strain differentiation are in progress using biotechnological methods (Leslie, 1991, 1993; Mansuetus, 1993; Zhang *et al.*, 1994). Similarly, molecular markers are being used to tag drought tolerance traits (Tunistra *et al.*, 1993; Rosenow, 1994). The application of molecular genetics with classical plant pathology and plant breeding should work together to develop new strategical approaches to solve these problems.

Charcoal rot. Although much progress has been made in breeding for charcoal rot resistance by using the nonsenescence trait (Rosenow, 1984; Pande and Karunakar, 1992), new information indicates that additional criteria for resistance to charcoal rot should be used (Tenkouano *et al.*, 1993). Mechanisms of resistance to *M. phaseolina* are still unknown, although research on those traits is being conducted (Frederiksen, 1993). The relationship of nonstructural carbohydrate (NSC) partitioning and charcoal rot resistance in sorghum was investigated by Tenkouano *et al.* (1992). These authors suggested that high yielding cultivars resistant to *M. phaseolina* could be developed since the developing grain could not be identified as the cause or the beneficiary of stem NSC exhaustion. So far, most of the unaffected hybrids tolerant to charcoal rot yield nearly as well as the unaffected susceptible ones. These observations are encouraging for improving sorghum for this trait.

Different biotechnological approaches are currently being used to detect variability among *M. phaseolina* isolates (Chase *et al.*, 1994). Knowledge of pathogen variability would be useful in breeding programmes. In general, similar approaches to Fusarium root and stalk rot resistance might be used in studies on control of

charcoal rot. Plant response to disease by induction of a large array of host response genes and modification of the expression of these genes could lead to enhanced disease resistance. This approach has recently been investigated and might also constitute a strategy to improve resistance to charcoal rot (Cui *et al.*, 1994; Nelson and Bushnell, 1994).

CONCLUSIONS

In summary, *F. moniliforme* and *M. phaseolina* have different requirements of temperature and soil moisture conditions for disease establishment and development. However, diseases caused by both pathogens are induced if stress conditions occur at anthesis or post-flowering. Complex traits are involved in the genetics of resistance to both pathogens, while selection procedures are troublesome and the results sometimes inconsistent. Effective and faster improvements of these traits may be done by applying molecular technologies. Attempts should be made to tag the different genes associated with the resistance to sorghum stalk rot diseases and to use linked RFLPs, for instance, to pyramid these genes into a single line. Resistant gene combinations may contribute to the durability of resistance (Kolmer *et al.*, 1991; Mundt, 1991; de Wit and van Kan, 1993).

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