ACTA MYCOLOGICA Vol. 33 (1): 109-121 1988

Some characteristics of isolates of *Rhizoctonia solani* from patch of wheat and barley

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Furgal-Wegrzycka H., Adamiak J., Adamiak E.: Some characteristics of isolates of Rhizoctonia solani from patch of wheat and barley. Acta Mycol. 33 (1): 109-121, 1998.

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Key words: Hordeum vulgare, Triticum aestivum, Rhizoctonia solani, Thanatephorus cucumeris.

INTRODUCTION

"Rhizoctonia root-rott" is an important disease of wheat (*Triticum aesti*vum) and barley (*Hordcum vulgare*) in Australia, Japan, Canada, South Africa, the United States and has been reported in Europe (Moen and Harris 1985: 0 goshi et al. 1990; Carling and Summer 1992; Carling 1996).

"Rhizocionia bare patch" disease of cereals, legumes and mixed legume--grass pasture was recorded in South Australia more than 60 year ago (R o v ir a et al. 1986). Since when, it has been recorded in most cereal--growing regions in other parts of the world. The disease occurred as patches of stunted plants in fields of wheat and barlev. Roots of diseased plants either were gridled (circumscribed) or rotted off (severed) by infections than began as sunken brown lesions. These root symptoms, and the ocurrence of the disease as the patches of stunted plants, match the symptoms of bare-patch or purple patch of cereals described in Australia and possibly barley stunt disorder in Socialand and crater disease of wheat in South Africa (Mur ar y and Nicolson 1979; Mur ay 1981; Deacon and Scott 1985; Burton et al. 1988).

All isolates of *Rhizoctonia* cultured from the roots of lower stems of diseased wheat and barley plants to botimed from the patches were multinucleate and the young vegetative hyphae exhibited morphological characteristics typical of *Rhizoctania solani* Kiban — telecomorph *Hamatephores cucumeris* (Frank) Donk (Parameter and Whitney 1970; Sneh et al. 1991).

R. solani anastomosis group 8 (AG-8) is the primary pathogen associated with bare patch, but AG-4 and AG-2-2 also are found on cereal roots (Moen and Harris 1985; Ogoshi et al. 1990; Carling and Summer 1992: Carling 1996). At least five distinct zymogram--pattern groups (ZG) exist within R. solani AG-8: There is good agreement between ZG and AG, although some of AG may by subdivided into more than one ZG (Mac Nish et al. 1993). Additionally, Mac Nish and Sweetingham (1994) established that from each distinct bare patch only a single ZG of R. solani AG-8 can be isolated, and they proposed that each patch emanates from a single infection focus. They also established that only R. solani AG-8 is responsible for Rhizoctonia bare patch disease, whereas isolates of other AGs of R. solani, as well as other Rhizoctonia species may cause varying levels of root-rot of cereals without producing the characteristic bare patches. Rhizoctonia bare patch caused by R. solani is more severe in cereals and other crops sown by direct drilling (no till) than where sown after conventional cultivation (Moore 1983; Rovira 1986; Welleru et al 1986)

With the growing acceptance of conservation tillage, which includes direct of tilling or no-till, by farmers in many parts of the world as well as in Poland it is likely that crop losses from root-rot caused by R solani will increase. This will lead to search for control by chemicals and resistant varieties and control by such means could be facilitated by having a better understanding of the anastomosis groups within R. solani that caused of "Rhizoctonia bare patch" (G ol e b n i ak et al. 1993).

Although R. solari can be easy isolated from diseased wheat and barley roots, determination of the AGs is diffault. A simple method to determine the AG would assist in understanding the ecology of the pathogen and the epidemiology of the disease. Current methods for determing the Rhizoctonia bare patch and the intraspecific groups of R. solari involve pathogenicity test, izozyme analysis, determination of the AG and DNA analysis of pure cultures (Sneh et al. 1991; Burpee and Martin 1992; Carling and Summer 1992; Carling 1996).

The objective of this study was the characterization of wheat and barley bare patch isolates of *Rhizoctonia solani* by anastomosis techniques.

MATERIALS AND METHODS

Is o lation and sources. Field isolates of *Rhitzentmia* spp. were recovered from surface settifized root segments of wheat and barley plants within and adjacent to three bare patches in a field in Experimental Station Balcyny in the years 1994–1996. The three patches were approximately 100–200 mapart from each other. Discased plants were taken from the centre and periphery of the patches. Apparently healthy plants were taken from outside the patches. Plants samples were obtained from the experimental fields under the corp rotation: sugar-beet-spring wheat athele manurel and four levels of mineral nitrogen (0, 0, 60, 60, 102 Nihetares, Spring wheat cultivar fmgat was cropping as follows: 1994 — wheat, 1995 — sugar beet following wheat, 1996 — spring barley following wheat.

The crop rotation was established in 1994 year. Rhizoctonia spp. were isolated from diseased plant using standard procedure.

Selective media.

1 - Ko-Hora medium amended: benomyl - 500 mg/1, prochloraz - 500 mg/1 (Ko and H o r a 1971).

2a - 2% water agar (WA) amended with ethanol - 20 ml/1, NaNO₃ - 5 g/1, KH₂PO₄ - 0.5 g/1 (Trujillo et. al. 1987).

Both media were dispensed at approximately 12 ml per Petri dish (PD) (e m in diameter). The dishes with three selective media were used for plant samples asay. Tissue sections 1-15 cm long were cut from the margins of lesions, washed under tap water, rinsed with an antibiotic solution (10 mg chloramphenicol and 100 mg of neomycin per 11 of distilled water) and transferred aseptically to Ko-Horn medium and to two other selective media. After the tissues had been grown for 3 days at $21-22^{\circ}$ C in the dark, cultures were examined at 400 × 50 mvedia of *R*. soloni.

N u c l e a r s t a i n i n g. Cultures on PDA were stained by a rapid technique with 0.5% aniline blue and by a HCL-Giernsa nuclear staining procedure, which to allowed count of nuclei in vegetative cells (H e r r 1979). H y p h al a n a st o m o s i s was observed on agar – coated sides. All the root isolates were paired with the nine tester isolates belonging to nine AGs group (AG-1 to AG-9 and GAG-1 (R. cervalis) to determine their affinities. Tester's isolates were paired in all possible combinations with representative isolate from diseased plants of wheat and barley. GAG-1 (R. cervalis) tester isolate was provided by Prof. Burpee (Canada), AG-6, 7, and Bb be' to Copshi (Japan) and AG-1, 2, 3, 4, 5) be't Carling (USA).

a Growth rate and characteristics of colonies. Ager disc (5-7 mm in dim) cut from margin of actively growing colonies on PDA were transferred to PD containing 15 ml of PDA freshly prepared from potators. Three disks of cachi solate were inclusted at 22–237C in the dark. Two measurements at right angles were taken and the increase in colony diameter between 24 and 48 hour of growth was recorded. The morphology and colour of colonies were compared during the first week of growth and later 24 days.

 P_i at h o g e n i c i ty t e s t s. Oat kernel inoculum was prepared by autochaving (100 ml of whole out kernels and 50 ml of distilled water in a 250 ml Erlemmyer flask for 1 hour on two consecutive days. Colonised agar disse of disalte tested were transferred to flask and inclusted at a 23 – 26° for 14 – 21 days prior to use. Seeds of wheat and barley were surface sterilized in a 95% ethyl alcohol for 30 seconds, then rinsed whit sterile water and planted wet on sterile sand in a plastic pot 15 cm diam. Five replicate pots were used per isolate. Seeding were inoculated by placing one infected oat kernel 1 cm below the sand in contact with the coleoptile. After 21 days, plants were washed free of alberings and and rated for devolpment of lesions.

The ability of isolates of *Rhizoctonia* sp. obtained from diseased plants to cause root-rot was tested using inoculum layer technique. Plastic pots with sterili sand as described above were used. A completely colonized 2% WA layer inoculum from a PD culture was placed to cover the top of the sand. A noncolonized agar layer was used for the control. Seven surface-sterilized wheat and barley seeds were arranged on top of the agar inoculum and then covered with SD in of the sand. Five replicate pots were randomized and plants were maintained at 10–25 C. Seedlings were washed free of adhering sand and both root length and fresh weith of tons were recorded.

A g a $r_{\rm Pl}$ la te vir u le n ce a s a y. Seeds were washed in a 0.3% solium hypothorite solution in deionized water for 3 minutes, rinsed in deionized water and air-dried before use. The seeds were placed in a circle l com form the edge of a 1.5 cm diam, steriel, disposable PD 15 cm in diam, containing 20 mi of 1.5% WA. About 1 mm diam, mycelial disc from the edge of a 2-3 day old 1.5% WA calture of each of the isolates was transferred aseptically to the center of each disc (one/disc). Control plates have non-colonized agar dishes.

Two days after inoculation, 3-5 drops of sterile distilled water were dispensed aspirally onto each seed. Dishes were sealed at two points with clear adhesive tape and incubated in continuos darkness at room temperature (of 5 days. Dishes were than placed in a laboratory and opposed to light for 12 hours (one day). The percentage of seedlings with infected roots and/or soleoptile and disease severity on individual seedlings was recorded by days after inoculation. Disease severity was rated based on a 1–5 scale: 1 = no symptoms. normal root development; 2 = localized lissicoloration without nercosis, near-normal root development; 3 = localized lissicoloration without nercosis, near-normal root development; 4 = nearly complete root-necrois, near-normal root gevelopment; 3 = localized lissicoloration here is were restricted. A disk containing 10 seedings represented one replication. The experiment was conducted four times, with each repetition in time representing one block of randomized complete design.

RESULTS

Results obtained in this experiment indicated that R. solani was the cause of *Relizocianis* hare patch², a root disease of wheat and barley. At least three species of fungi in the *Relixocianis* hare patch² complex may induce root-root of wheat and barley. For this study three patches in a wheat and barley were chosen. The patches were in a roughly triangular formation with centers about 10 m apart and was first observed usually at the end of the growing seasons. For each patch the isolates of R. solani were first anastomosed with other isolates collected from the same patch and next were anastomosed with isolates collected from the same patch and next were and from each the patches were clones, or whether the same clone colonized the three patches. This was also done to determine which species or anastomosid with stunting of wheat and barley amended with different kinds of organic market.

Detailed descriptions of the categories of anatomosis reactions in *R* solari are given in Table 2 after C a r l i n g and S ur m e r (1992). These categories range from C_0 in which no reactions occur to C_0 in which walls and membranes of anastomosing hyphae fuse and the cytoplasm of nastomosing hyphae intermingles. The C_1 reaction is comparable to the perfect reaction. The C_3 reaction would be expected to occur in selfanastomosis reactions used to determine clonal relationship in all AGs of *R*. solari. Result obtained in this study indicated that only isolates of R. soluril AG-8 collected from "*Rhizectonia* bare patches" were members of the same clone as indicated by the clonal anastomosis reaction. Anastomosis reactions also indicated that all isolates collected from the same fael were members of the same clone.

There were differences in the patterns of isolates of *Rhizoctonia* sp. from the centre, periphery and from outside of the patches. *R. solari* anastonosis group 8 was associated with diseased plants collected from inside of the bare patches. *R. solari* anastonosis groups AG-4 and AG-2 were associated mainly with diseased plants collected from peripheries of the patches. Isolates of *Waiters* ap. and binucleate *Rhizoctonia* sp. were isolated more frequently from periphery of patches and from adjacent of the healthy plants.

Twenty nine percent of the isolates of R. solar obtained from diseased roots of wheat and bardy were AGS, 425 $^{\circ}$ AGA, and 38 $^{\circ}$ AG-22. The remaining were: AG-3 (two isolates), AG-5 (one isolate), R. zear (sixteen isolates), AGS made up 60 $^{\circ}$ of the isolates of R. solarify of the center of the patches, whereas AG-4 and AG-2-2 made up 80 $^{\circ}$ of the isolates from the outside of the patches.

All isolates of *R. solani* were pathogenic on wheat and barley seedlings in laboratory test experiment. Pathogenicity test on wheat and barley indicated that all binucleate isolates of *Rhizoctonia* sp. and *Waitera* sp. were mildly virulent or avirulent. Isolates of *R. zeae* were all pathogenic on wheat and barley.

Isolates of AG-8 from the patches could be separated into three groups on the basis of their pathogenicity on wheat and on barley seedlings: those that caused no disease, those that caused severe root-root and those of intermediate virulence. Highly virulent isolates of AG-8 were found only within the patches, whilst other two distinct groups were found both outside and inside the patches. The difference between two distinct groups of AG-8 was not associated

The difference between two distinct groups of AG-8 was not associated with differences in morphology of cultures or growth rate on PDA. Both types had the same growth rate and morphology on PDA.

CHARACTERISTICS OF CULTURES

The characteristics of cultures of the anastomosis groups of Rhicotomia solari and Rhicotomia are, which were isolated from diseased roots of wheat and barley are given in Table 1. The AG-3 isolates were all identical on PDA: the colonies were whithis that first and tured to light brown after two weeks and showed concentric zonation. Sclerotia were not distinctive. On Czapk-Dox agar, the colonies of the AG-3 isolates became brownish with abundant aerial mycelia. The growth of AG-3 isolates on agar media were slower than that of isolates from other anastomosis groups.

Category	Relatedness	Description of interaction
C ₀	Not related (different anastomosis groups)	No interaction
C	Distantly related (different anastomosis groups or same anastomosis groups)	Contact between hyphae; apparent con- nection of walls but no evidence of wall penetration or membrane-membrane contact; occasionally one or both ana- stomosing cells and adjacent cells die
C2	Related (same anastomosis groups, dif- ferent clones)	Wall connection obvious; membrane contact uncertain; location of reaction site obvious; diameter of anastomosis point less than hyphal diameter; ana- stomosing and adjacent cells always die
C3	Closely related (same anastomosis group), (same clone), (same isolate)	Wall fuse; membranes fuse; anastomosis point frequently not obvious; diameter of anastomosis point equal or nearly equal to hyphal diameter; anastomosing cells and adjacent cells may die but generally do not

Table 1

Categorization of anastomoses in Rhizoctonia solani occur between hyphae*

⁶Categorization based reactions and cytological occurrences at the point of anastomosis between two hyphae (HYPHA-HYPHA reaction). These occurrences are observable at the microscopic level. Test was created by Catrling and Lenir in 1992.

Some isolates of R. solani collected from diseased roots of wheat and barley in cropping experiment, were recently found to constitute what we believe to be an undescribed anastomosis group caused coleoptile rot on wheat and were less pathogenic on barley seedlings. These isolates caused lesions on the coleoptile of wheat. Three of these isolates were pathogenic to barley seedlings. These isolates of R. solani obtained from field experiments were indistinguishable from one another in terms of anastomosis reactions and constitute a separate anastomosis group. All the isolates of separate anastomosis group were multinucleate and probably belonged to the indigenous population of R. solani. The indigenous isolates of R. solani grown on PDA were white to light tan when young after but after 3 weeks ranged from brown to dark brown. A few isolates had yellowish pigmentation. Concentric rings of dark and light mycelium were visible in most cultures and this zonation was visible from early stages of development. Mycelium was floccose in early stages of growth, but as cultures aged mycelium became increasingly appressed to the agar surface. Sclerotia generally ranged from few to many and were 0.5 to 2.0 mm in size. Individual sclerotia often coalesced into large clumps. Mature sclerotia were tan to light brown and were scattered randomly over the agar surface

The diameters of mature hyphae of the isolates examined ranged from 7.1 to 8.6 μ m. The number of nuclei the cells ranged from 4 to 7 per cell.

In thiamine requirement test the isolates of $Acce^{-1}$ and $Acce^{-1}$ percenting thiamine prototrophic ACs of *R*. solari and grew at approximately the same rate with or without the thiamine. Isolates of $Acce^{-1}$ percenting that auxotrophic ACs. The rate of growth of evaluated separate indigenous anastomosis group of *R*. solari were the same with to without of thiamine.

DISCUSSION

Rhizectonia solari was the cause of "Rhizectonia bare patch" a root disease of wheat and barley in field experiment conducted in the years 1994–1996. R. solari anastomosis group AG-8 was the primary pathogen associated with bare patch, but AG-4 and AG-22 also were found on roots of wheat and barley. All the pathogenic isolates of AG-8 have been collected every year from inside the bare patches whereas isolates of AG-4 and AG-22 have been recovered mainly from outside the patches. AG-8 was the dominant species recovered mainly from outside the patches. AG-8 was the dominant species recovered from wheat and barley plants chibiting symptoms of root-rot, but 65% of the R. solari isolates recovered from wheat and barley belonged to AG-4 and AG-22.

Although R. solari anastomosis groups AG-4, AG-3-22 and AG-8 were isolated at similar frequencies from wheat and barley roots and both incite root-rot, comparative pathogenicity studies indicated that R. solari AG-8 was the relatively more important of the three pathogens under increased use of minimum tillage system or amended the microplots with straw and with high levels of mineral nitrogen.

The isolates of AG-8 examined in this study could be placed into two distinct groups: pathogenic on wheta and barley and sprophytic. Isolates of *R. solari* AG-8 collected from "*Rhitzectonia* hare patches" also characterized by anastomosis technique. Anastomosis technique demonstrated that multiple isolates of AG-8 from the same bare patch or from different the patches were members of the same clone. "*Rhitzectonia* hare patch" disease of cereals, legumes and mixed legume-grass pasture was first recorded in South Australia more than 60 years (M a c N is h 1985; R o b err is and S i va s it h am p ar a m 1986; R o vir a et al. 1996; Y an g et al. 1994). Since than it has been recorded in most cereal-growing regions of South Africaof the world (M u r ray and N i col s on 1979; M u rray 1981; O e a c on and S c ot 1 1985; M o e n and H ar ris 1985; C g o s h i et al. 1990). *Rhisoctonia* root-root of wheat and barley is an important disease also in Poland (P o ka c & a and W o it as z < k 1977, 1976; Truszkowska et al. 1979, 1983; Mańka et al. 1983; Truszkowska et al. 1983; Łacicowa 1985; Weber and Zdźiebkowski 1989).

In recent years the incidence of the disease has increased with the increased use of minimum tillage systems. *Rhiteconiai* root-ord of wheat and barley is an important disease especially where these cereals are grown in no ill or direct-drill management system or in crop rotation with polatoes, sugar, beet and some legumes as lupine. *Rhiteconiai* root-ort of wheat and barley is an important disease in long term monoculture (T us x k ows k = at 1.979, 1983; M oor 0×1983 , R oor 0×173 and V en 1985; W ell cr et al. 1986; H i de and F ir n a ger 1990; L u ca s et al. 1995; R u sh et al. 1997, C arr in g 1996.

R. solari anastomosis group AG-8 and R. oryzae R.y.ker et G oo ch are widely distributed in USA. Australia South Africa and United Kingdom and both are capable of causing this disease (B u r t o n et al. 1988; S n ch et al. C a r l in g. 1996). In Europe, Canada and Japan R. solari AG-8 and R. oryzae were the dominant species and intraspecific groups of Rhitzetonia recovered from wheat and barley plants exhibiting symptoms of root. On the other hand about 85% of R. solari JoSaer recovered from wheat or barley-field soil belonged to AG-3, AG-4, AG-5, AG-22, AG-9, AG-10. In USA especially in Texas R. solari AG-4 was the dominant Rhitzetonia species recovered from wheat and isolates of AG-4, AG-5 AG-11 were capable of causing significant post-emergence root-root of wheat. (M o en and H a r r is 1985; O g o s h i 1987; O g o s h i et al. 1990; C a r l ing a 105 S um m er 1992; C a r l ing 1996).

Although several anastomosis groups of R. solard are isolated at similar frequencies from wheat and barley roots comparative pathogenicity studies indicated that R. solard AG-8 is the relatively more important of the pathogenes under the growing conditions encountered in USA. Australia and in Europe. (S ne h et al. 1991; C arl 1 in g and S un mer 1992; C ar I in g 1996). M ac N is h and S w e ct in g h am (1994a) established that only R. solardi AG-8 is responsible for Rhizoctonia bare patch disease, whereas isolates of other AGs of R. solard is well as other Rhizoctonia species may cause varying levels of root-rot cereals without producing the characteristic bare-patches.

The mechanism of patch formation is not known. The isolates of R solari, which are most commonly associated with cereal patches, differ in their morphology and pathogenicity (0 g o sh i 1987; S ne h et al. 1991, B ur pee and M artin 1992; C arl in g and S um mer 1992; C arl in g 1996, Iraditionally, isolates of R. Sadari collected from the bare patches have been grouped into anastomosis groups (AG) and more recently into peetic zymogram groups (ZG) on the basis of the peetic enzymes produced during growth on pectin medium. (Parameter and Whitney 1970; Mac Nish et al. 1993). There is good agreement between ZG and AG, although some of the AGs may be subdivided into more than one ZG. (Mac Nish and Sweeting ham 1994b). At last fire distinct zymogram – patter groups (ZG) exist withing *X* solari AG-8. Additionally Mac Nish and Sweeting ham (1994b) established that from each distinct bare patch only a sing ZG of *R*. solari AG-8 can be solated, and they proposed that each patch emantes form a single infection focus. Mac Nish and Sweeting ham (1994b) concluded, on the

M a c N is h and S w e e i in g h a m (1994b) concluded, on the basis of anastomosis and pectic zymogram grouping, that each patch is dominated by a single clone of *R*. solard, and that patches with the same zymogram group in close proximity are more likely to be dominated by the same clone than are patches separated by greater distances. This implies that single clones may not be confined to the area of the patch, but can extend beyond this area, although patches will only occur when the balance of conducive and suppressive factors in the soil is favourable.

However in contrast to these results Y a n g et al. (1994) found that isolates from the same patch, although of the same AG and 2G, could be grouped on the basis of pathogenicity tests into highly virulent (W1, weakly virulent (W2) and those of intermediate virulent (H2). While IV and W2 types were found both outside and inside the patches. HV strains were found only within the patches. Furthermore, vegetative compatibility tests showed that isolates with different mating type genes could be found in the same patch.

Banne parks. Recently the bare patches strains of R, solani are characterized by use the molecular methods (M a z 0 11 a et al. 1996; C a r 1 ing 1996). Previous studies have shown that techniques that detect polymorphisms in DNA (random amplified polymorphic DNA (RAPD) by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFL) Preveal that there is a much greater level of variability between strain fram can be detected by AG or ZG (typing of AG-8. Characterization of have patch strains using, these techniques would generate significant information regarding the variability of strains from the same patch, the relationship between strains from adjacent patches, and the role that these strains play in patch formation. Y an g et al. (1995) characterized or certael bare-patch isolates of

Y a n g et al. (1995) characterized of certal bare-patch isolates of $R_{\rm S}$ solar by RADP-DCR analysis and the yound that isolates of AG-8 from the patches gave very similar RAPD-PCR patterns with all primers tested. There was not difference between isolates from inside the patch compared to those from outside. All tested isolates of AG-8 are generalized very similar belonged at least to two distinct ZG (ZG-1 and ZG-2) and differ only in pathogenicity in finances. observed among isolates of AG-8 collected from the patches indicates that this anastomosis group is very diverse genetically. Parasitic and saprophytic fitness are complex traits and likely to be controlled by several genes and may involve the same or different genes (M a z o 11 a et al. 1996; C a rling 1996).

Brisbane et al. (1995) were used RAPD-PCR analysis to generate polymorphism of R. solani isolates of anastomosis groups of AG-8 and AG-4 infecting wheat in Australia and other countries. The isolates of R. solani AG-8 examined in their study could be placed in two distinct groups: those that caused no disease and those that caused severe root-rot of wheat. The polarity in the disease-causing capabilities of these two groups of R. solani AG-8 suggests that the two groups might be genetically distinct. Ribosomal DNA sequence data have been used to examine phylogenetic relationships among these two distinct groups. Preliminary studies based on the sequence of the ITS of the rDNA region confirm that isolates grouped together based on ability to anastomose can be quite different and that isolates of R. solani AG-8 can be placed into at least two evolutionary groups (8-1 and 8-2). Indeed, the nonpathogenic isolates of R. solani AG-8 (8-2) may be biologically and perhaps phylogenetically more similar to isolates of R. solani AG-6 than the pathogenic isolates of R. solani AG-8 (8-1).

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Cechy izolatów Rhizoctonia solani ze zgorzeli pszenicy i jeczmienia

Streszczenie

Z porzdowych rolim posewiej i jezomienia z objawami zgorzeli korzeni i podztawy Adriba wysobenione wiejdowie d wiejdowe o todzy Ableczenia w p. Porzyakacieka čludate do grup zgodność u węztatywnej czaszczona na podztawie zdołność do tworzenia heterokariosów z tesriemi. Twiezdowa, ke owa zgorzed wysoływam bię tperzewiejdowie z testofa *Beizeniani* adrawi klima nadające do grup zgodność węztatywnej AGA, AG-2-21 IAG-4. Ustały *R. soda* adrawi klima nadające do grup zgodność węztatywnej AGA, AG-2-21 IAG-4. Ustały *R. soda* podziele za rozwie za rozwie z rozwie z rozwie za rozwie podziele rozlim za rozwie z rozwie z rozwie. Beizenomia