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Research Article

Simple sequence repeats to identify true hybrids in bacterial wilt susceptible X resistant crosses in groundnut

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Abstract:
Bacterial wilt of groundnut is a devastating disease in China and Southeast Asia. Planting resistant cultivars is considered the most effective for disease management. Transfer of resistance to high yielding adapted groundnut cultivars is urgently needed. Identification of true hybrids in susceptible × resistant crosses is a must as it is of relevance to resistance genetics and breeding. In this study, simple sequence repeat primers coupled with DNA template prepared from 3-5 mg cotyledonary tissue and a 30 min fast silver-staining procedure were successfully utilized to discriminate true hybrids from selfs in 24 cross combinations. 144 (33.41%) out of 431 seeds were identified as hybrids, with percentage of hybrids ranging from 8.33%-100%.
Keywords: groundnut, hybrid identification, SSR, bacterial wilt

Introduction:

Bacterial wilt (BW) caused by *Ralstonia solanacearum* (Smith) Yabuuchi **et al**. is a great threat to groundnut (*Arachis hypogaea* L.) production in China and Southeast Asia. At present, desirable methods for effective chemical control of the disease are unavailable, and planting resistant cultivars is regarded as the most effective and economical means to disease management (Wang et al., 2009). A large number of resistant germplasm accessions have so far been identified; unfortunately, most of them have low yield potential (Wang et al., 2009). In Shandong province, the leading groundnut producer of China, where large seeded groundnut varieties dominate, yield potential is the main factor influencing growers' decision on a cultivar, even in diseased regions, Linyi and Rizhao. Reportedly, groundnut area severely affected by BW in the two cities amounted to 35 000 ha (Zhang et al., 2008). Transferring BW resistance to high yielding adapted groundnut cultivars through hybridization therefore becomes an urgent task. For breeding and genetics purposes, true hybrids in these crosses need to be identified, preferably soon after harvest of F_1 seeds.

Groundnut breeders used to identify true hybrids and selfs by differences in morphological features, disease reaction and quality traits between F_1 and parental plants, or by segregation in F_2 and subsequent populations (Chen et al., 2009). Recently, molecular markers, in most cases simple sequences repeats (SSRs), have been used in identification of F_1 hybrid groundnut plants (Gomez et al., 2008, Chen et al., 2009, Li et al., 2009). Currently available protocols for DNA template preparation from groundnut seeds are either destructive or time-consuming (Chenault et al., 2007, Hu et al., 2009), except the one reported by Yu et al. (2010), where 3-5 micrograms of groundnut cotyledonary tissue and 30 min are enough for at least 20 polymerase chain reactions (15 µl total volume per reaction). In the present report, Yu et al. (2010)'s protocol was successfully used to identify true F_1 hybrids from BW susceptible × resistant crosses in groundnut.

Material and methods

Groundnut material**:** Seven groundnut lines/cultivars with BW resistance were used as male parents, and 12 high yielding groundnut genotypes were used as female parents (table 1 and table 2). A total of 24 cross combinations were made (table 2). Artificial hybridization in groundnut was carried out according to the standard procedure (Yu et al. 2011).

DNA extraction: DNA templates for PCR were extracted from cotyledonary tissue of parents and

the resultant hybrid seeds following the method described by Yu et al. (2010).

SSR analysis**:** SSR primers were synthesized based on He et al. (2003), He et al. (2005), Moretzsohn et al. (2005), Jiang et al. (2007) and Wang et al. (2007). SSR analysis was conducted in 3 replications. The PCR mixture (15 µl total volume) consisted of 2µl of DNA template, 0.6 µl of forward and reverse primers (10µM) each, 1 µl of $MgCl_2$ (25 mM) and 7.5 µl of Tiangen 2 × *Taq* PCR Master Mix (Tiangen Biotech, Beijing, China). PCR program was 10 cycles of denaturing at 95 ºC for 1 min, 65ºC for 1 min (1 ºC decrease per cycle), and 72 ºC for 90 sec, followed by 30 cycles of 95 ºC for 1 min, 55ºC for 1 min, and 72 º for 90 sec.

Bands were separated on a 6% denaturing polyacrylamide gel. Silver-staining was done based on protocol of Liang et al. (2008) with minor modifications. Afterwards, washed with distilled water for 2 times (1 min), the gel was placed in fixation and silver-staining solution containing 1% glacial acetic acid, 10% ethanol and 0.2% $AgNO_3$, for 10 min. Then the gel was washed again with distilled water for 2 times (2 min), and transferred to developing solution (3% NaOH, with 1 ml formaldehyde added in 200 ml total volume just prior to developing). Thirty minutes were generally enough to develop clear bands.

Results and Discussion

Totally 18 SSR primer pairs were found informative (He et al. 2003, He et al. 2005), and were used to identify true hybrids in the 24 cross combinations (table 2). Fig. 1 and fig. 2 showed the banding patterns in 2 of the cross combinations, where male parent produced a characteristic band absent in female parent, and the resultant "F_1" seeds with the band were identified as hybrids and those without the band as selfs. All the cross combinations and replications with 2 primer pairs gave consistent results, demonstrating that the present protocols were feasible and reproducible.

Out of 431 seeds, 144 (33.41%) resulting from the 24 cross combinations were identified as hybrids, with percentage of hybrids ranging from 8.33%-100% (table 2). In most part, this is not a reflection of difference in ease of hybridization, but rather, is a reflection of the performance of the operators. Lower percentage of hybrids may be ascribed to poor hand crossing operations. Hybrid identification by SSRs therefore will be useful to advance only hybrid progenies instead of selfs which in turn save the cost, time, labour and area of experimentation.

Acknowledgments

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Table 1 Parental lines/cultivars used in hybridization

Parents	Disease reaction to Bacterial wilt	Salient features
R87	HR	*Arachis glabarata* derivative with small seeds
R106	HR	*Arachis glabarata* derivative with small seeds
R15	HR	*Arachis glabarata* derivative with small seeds
R16	HR	*Arachis glabarata* derivative with large seeds
R1	MR	*Arachis glabarata* derivative with large seeds
Quanhua 646	R	Small-seeded cultivar
Quanhua 10	R	Small-seeded cultivar
LF2	S	Large-seeded line
Huayu 22	S	Large-seeded cultivar
Huaxuan 10	S	Large-seeded cultivar
Qunyu 101	S	Large-seeded cultivar
Luhua 10	S	Large-seeded cultivar
Huayu 33	S	Large-seeded cultivar
Huayu 34	S	Small-seeded cultivar
Huayu 31	S	*Arachis glabarata* derivative, Large-seeded cultivar
Huayu 20	S	Small-seeded cultivar
Tieling Silihong	S	Small-seeded landrace
09-L36	S	Large-seeded *Arachis glabarata* derivative with cold tolerance
09-L43	S	Large-seeded line

Note: HR=highly resistant, MR=moderately resistant, R=resistant, S=susceptible.

Table 2 Results of hybrid identification in 24 cross combinations by SSR

Cross combination	No. of F_1 seeds obtained after crossing	No. of true F_1 seeds	% of F_1 seeds	Polymorphic SSR primers
LF2×R87	5	1	20.00	PM53, S23
Huayu 22×R87	10	2	20.00	PM53, S23
Huaxuan 10×R87	24	2	8.33	PM53, S23
Qunyu 101×R106	11	5	45.45	PM35, PM39
Luhua 10×R106	14	5	35.71	PM35, S23
Huayu 33×R106	9	6	66.67	AC2B5
LF2×R15	20	4	20.00	PM145, S21
Huayu 22×R15	24	7	29.17	S19, S20
Huaxuan 10×R15	15	8	53.33	PM145, S21
Qunyu 101×R16	34	13	38.24	PM145, S29
Luhua 10×R16	28	5	17.86	PM145, S29
Huayu 33×R16	21	13	61.90	S23, S28
Huayu 34×R1	20	10	50.00	PM 137, S20
Huayu 31×R1	14	14	100.00	S18, S19
Huayu 20×R1	30	3	10.00	S20, 7G02
Tieling Silihong×R1	16	3	18.75	PM137, S19
09-L36×R1	24	3	12.50	S12, S19
09-L43×R1	23	12	52.17	S20, S23
Tieling Silihong×Quanhua 646	10	3	30.00	S5, S14
09-L36×Quanhua 646	20	5	25.00	S5, S14
09-L43×Quanhua 646	24	9	37.50	S5, S15
Huayu 34×Quanhua 10	5	2	40.00	AC2B5
Huayu 31×Quanhua 10	20	3	15.00	PM137, S18
Huayu 20×Quanhua 10	10	6	60.00	PM137, 7G02
Total	431	144	33.41	

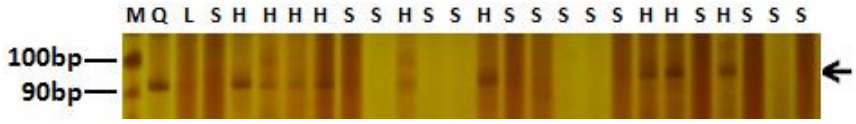

Fig. 1 Primer pair S15 identified true F_1 hybrid (H) and selfs (S) resulting from 09-L43×Quanhua 646, arrow indicating a characteristic band present in Quanhua 646 (Q, male parent) but absent in 09-L43 (L). M: 10bp DNA Ladder (Invitrogen)

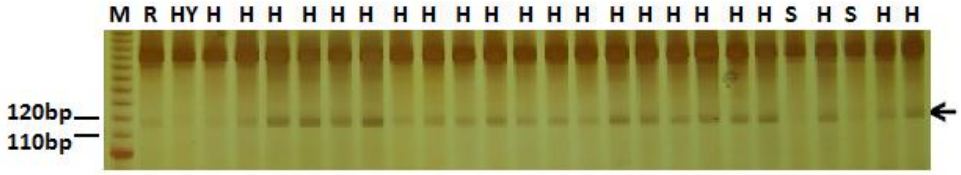

Fig. 2 Primer pair S20 identified true F_1 hybrid (H) and selfs (S) resulting from Huayu22×R15, arrow indicating a characteristic band present in R15 (R, male parent) but absent in Huayu22 (HY). M: 10bp DNA Ladder (Invitrogen)