Final Report

Title: Transfer of hydrogen uptake (Hup) genes from *Rhizobium leguminosarum* to fast-growing, salt tolerant *Rhizobium fredii*.

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Summary

The transfer of PIJ 1008 plasmid in *Rhizobium leguminosarum* 3894 (as a donor) to a spontaneous mutant Rif^R Tet^R (250, 10 ug ml^{-1}) of *Rhizobium fredii* HH 303 (Hup^-) was a complished by mating on 0.45 u m Millipore filter membrane. The filter was overlayed on non-selective Ty media overnight. The growing colonies were suspended in buffer and appropriate dilutions were plated onto selective Ty media (Rif_{50}, Kon_{50}. Tet_1 ug ml^{-11}) to counterselect the donor. The transconjugants were tested in greenhouse experiment for their ability to nodulate soybean. Four soybean cultivars namely: Lee, Crawford, Colombus and Kent were inoculated with the transconjugants. Data showed that Lee had the lowest nodule dry weight, while the highest nodule dry weight was found on Crawford. Several of the transconjugants exhibited increases in plant dry weight. The highest plant dry weight was observed by the recipient parent strain which exhibited a relatively low nodule dry weight. Most transconjugants displayed higher nodule dry weights than the recipient. The highest % N was found in Colombus and Lee plants inoculated with T#6 and T#12 respectively.

Field experiment of 1988 season indicated that nodulation of Clark plants by most of the transconjugants was better than of that Crawford plants. In general, inoculation with T_1, T_3, T_{13} and T_{22} resulted in the highest nodule dry weight values compared with the recipient strains HH 303 (Rif_{250}. Tet_{10}). Seed yields of soybean responded differently to inoculation with the transconjugants. The highest seed yield was found with T_{27}.

In 1989, inoculation with T_3, T_4, T_5, T_{10}, T_{11}, T_{14}, T_{18}, T_{17}, T_{19}, T_{25} and T_{28} had significant influence on nodule dry weight and N % in leaves or seeds of the two cultivars compared with the uninoculated plants. Inoculation with the transconjugants T_{14}, T_{17}, T_{25} and T_{27} significantly increased both pod and seed yields of soybean plants in comparison with the recipient strain HH 303 (Rif_{250} Tet_{10}).

Introduction

Soybean is relatively a new crop to be grown in Egypt. Because soybeans have never been cultivated on soils in Egypt, there exists no indigenous soil population of *Bradyrhizobium japonicum*. Hence, soils must be inoculated with *B. japonicum* prior to planting.

Survival of introduced strains of *B. japonicum* in Upper Egypt soils is partially limited by some environmental and soil factors such as a relatively high salt concentrations in soils. Because of poor drainage and upward movement of salts in the soil during evaporation, excessive concentrations of salts may accumulate on top of the soil and near the surface. The excessive salt concentrations in soil of the not zone are highly toxic to *B. japonicum*, and therefore necessiate the inoculation of seeds every year prior to planting, and even then, inoculation may not result in the formation of effective nodules.

The research described here aimed to transfer, by conjugation, genes that confer nitrogen fixation efficiency into a salt-tolerant, ineffective strain of *Rhizobium fredii*. Such transconjugant strain of *R. fredii* that nodulate soybean will be capable of surviving in saline soils and will fix large quantities of atmospheric nitrogen and thus potentially enhance soybean yield.

The proposed research took advantage of salt tolerance of the newly described group of fast-growing soybean rhizobia (Vincent, 1977; Jordan, 1982; Keyser et al. 1982 and Yelton et al. 1983). Because this group of soybean rhizobia has only recently been described, very little work has been conducted with it.

Two factors that involved the efficiency of symbiotic N_2-fixation at the bacterial level are: H_2 evolution by nitrogenase and H_2 oxidation by uptake hydrogenase (Evans et al. 1981; and Robson and Postgate (1980).

The evaluation of H_2 by nitrogenase is ATP-dependent and occurs concomitantly with the reduction of N_2 to NH_3. In the Rhizobium-legume symbiosis, 40 to 60% of the electron flux to nitrogenase may be evolved as H_2 (Schubert and Evans 1976). Dixon (1977) proposed three possible roles for up take hydrogenase system: the protection of nitrogenase from O_2 damage, the prevention of H_2 inhibition of nitrogenase, and the production of ATP to supplement the energy requirements for N_2 fixation.

The potential agricultural significance of energy loss from nodule through H_2 evolution was appointed first by Schubert and Evans (1976) who reported that the extent of loss in many nodulated legumes ranged from 40 to 60% of the energy flux through the nitrogenase system.

Many evidence have shown that the oxidation of H_2 by hydrogenase is benificial to N_2-fixing organisms. Albrecht et al. 1979 found that the nodules from soybean plants inoculated with Hup^+ strains lost little or no H_2 wherease nodules formed by the Hup^- inocula last H_2 rapidly. The dry weights and total N_2 contents in shoots of plants nodulated with Hup^+ strains averaged 16.6 and 26%, respective increases than those nodulated with Hup^- strains. The differences were significant at the 1% level of probability.

Many Rhizobium strains lack an uptake hydrogenase and nodules of plants inoculated with those organisms evolve large amounts of H_2. The traditional method for obtaining Rhizobium strains with favorable symbiotic properties has been through the natural selection of field isolates which produce superior growth of the host legume plant (Vincent, 1970). The more recent approach is to construct on improved *Rhizobium* strains by gene transfer. Brewin et al. (1980) reported that genetic determinants for hydrogenase activity (Hup^+) and nodulation capacity (Nod^+) were co-transfered between *Rhizobium leguminosarum* strains during conjugation. Dejong et al. (1982) found that plasmid PIJ 1008 carried genetic

determinants for an uptake hydrogenase activity (Hup^+) as well as nodulation capability (Nod^+ and Fix); this increased the effectiveness of strains carrying PI 1008.

The fast-growing *R. fredii* strain HH 303 is capable of effectively nodulating American varieties of soybean. However, because HH 303 does not contain the gene of hydrogenase activity, it evolves considerable quantities of hydrogen gas from nodules and thus the overall metabolic efficiency of this strain is very low compared with hydrogen up take positive slow-growing *B. japonicum*. To enhance its metabolic efficiency and nitrogen fixation, we intented to transfer hydrogen uptake determinants into this strain (HH 303).

Hydrogen uptake determinants (Hup) have previously been shown to enhance the nitrogen fixation efficiency of rhizobia (Albrecht et al., 1979; Brewin et al., 1982; Denarie et al. 1981; Beringer et al. (1982); and Dejong et al. 1982). The hydrogen uptake genes we proposed to transfer into soybean rhizobia are localized on a recombinant symbiotic plasmid which is self-transmissible. Fast-growing rhizobia exconjugants which acquire Hup determinants should be capable of retaining molecular hydrogen and thus should exhibit enhanced nitrogen fixation efficiency.

The objectives of this work were to transfer Hup genes from *Rhizobium leguminosarum* 3894 to *R. fredii* HH 303 and to test the transconjugants for nodulation and nitrogen fixation on some soybean cultivars under both greenhouse and field conditions in Assiut.

Materials and Methods

Parent strains:

The strain *R. fredii* has been kindly supplied by Dr. D.A. Phillips, University of California, Davis. Substrain of *R. fredii* HH 303 with multiple, high level drug resistance (Rifampicin and streptomycin) was used as the recipient of hydrogen uptake determinants from *R. leguminosarum* strain 3894. The hydrogen uptake determinants in *R. leguminosarum* strain 3894 are located on a recombinant, symbiotic plasmid which is self-transmissible. The plasmid, PIJ 1008, contains the In_5 konamycin resistance marker so that plasmid transfer can be monitored and selected for. DeJong et al. (1982) described the markers of PIJ 1008 as follows: Kan-r-Kanamycin resistant (6 ug ml^{-1}); Tra^+-transmisble by conjugation; Nod^+ nodulation function; Fix^+-nitrogen fixation function; and Hup^+-hydrogen uptake activity.

Transfer of Hup genes:

The actual transfer was accomplished by mating *R. fredii* HH 303 with *R. leguminosarum* 3894 on 0.45 Millipore membrane filter (Beringer et al. 1978). The doner and recipient were mixed in an approximately one to ten ratio. Following an over night mating, the my ture was vacum filtered. The filter was then overlayed on nonselective media for 12 hrs. At this time, the colonies were suspended in a Tween buffer to isolate individual cells.

Selection was made for transmissible kanamycin by plating the appropriate dilutions onto selective media (Kanamycin and rifampicin or tetracycline) to isolate the transconjugants.

Presumptive transconjugants were regrown on Ty media containing Rif_{50} Kon_{50} Tet_1 and maintained on the same selective media.

Confirmation

All transconjugants were pooled and the mixture was used to inoculate soybeans (Glycine max cv. peking and welliams) as a trapping system. After four weeks of growth, nodules were squashed and individual isolates were streaked onto selective Ty agar media (Rif_{50}, Kan_{50} Tet_{1}). Individual isolates were purified by three successive streaking for single colonies. All isolates designated HH 303-1 to HH 303-28.

Field experiments:

These experiments were carried out at the Experimental Farm Station, College of Agriculture, Assiut University in 1988, 1989 and 1990 seasons. Two soybean cultivars Clark and Crawford were used in this work. A randomized complete block experimental design was employed with four replications. Plots consisted of 6 ridges 3 m long and 0.6 m between ridges. Inoculated soybean seeds were drilled into holes (3 seeds/hole with 15 cm apart between holes). Plant samples were taken 70 days after planting. Dry weights of nodules and plants were determined. Dried plants were ground and N content was determined by the Kjldahl method (Black et al. 1965). At harvest plant samples were taken for pod and seed determination. Nitrogen seed content was also determined.

Preparation of inocula:

Transconjugants were individually grown on tryptone broth media supplemented with antibiotics rifampicin konamycin and tetracycline at concentrations of 250, 50 and 1 ug ml^{-1} respectively. Cultures were incubated at 28±2°C for 4 days. Aliquats were added to sterilized, acidity neutralized ground peat moss at the bases of 50% (V/W). The inocula were further incubated at 28±2°C for one week before use.

Seed inoculation:

Soybean seeds were wetted with 40% of arabic accacia gum solution. Then the seeds were mixed throughly with the inoculum at the rate of 10% of seed weight. The inoculated seeds were immediately planted and irrigated.

Results and Discussion

An antibiotic resistant mutant of *R. fredii* HH 303 was isolated by spreading aliquots of this culture on tryptone media (Beringer et al. 1978) supplemented with rifampicin and tetracycline concentrations of 250 and 10 ug ml^{-1} respectively. This mutant was designated as HH 303 Rif_{250} Tet_{10} and used as a recipient, while *R. leguminosarum* strain 3894 (Hup plasmid PIJ 1008) was used as a donor. After mating of both strains, the following media were used to counterselect the doner, Ty medium with: a) Kan_{50} Rif_{100} Tet_{10}, b) Rif_{100} Sr_5, Tet_{10}, c) Rif_{50} Sr_{50} Tet_1 and d) Kan_{50} Rif_{50}, Tet_5. No growth was observed on any of the above media. The membrane mating was repeated on Ty selective media (Rif_{50}, Kan_{50} and Tet_1 ug ml^{-1}). This selective media was used to counterselect the donor. Results in Table 1 showed that the transfer frequency was approximately one order of magnitude greater than the number of the recipient. The transfer frequency was found to be $2.3.10^{-8}$ with 145 of the total transconjugants.

Greenhouse experiment:

Results of the greenhouse testing of each of the transconjugants averaged together in each of the four soybean cultivars are presented in Table 2. It is evident the significant differences among the soybean cultivars. Kent was the highest cultivar in plant dry weight, while the lowest cultivars was Lee. The plant dry weight of Kent was over 30% greater than Lee.

Table 1. Frequency of transfer of plasmid pIJ1008 in matings of *R. leguminosarum* 3894 and *R. fredii* HH303 (Rif^R Tet^R)

Mating/Control	Selective medium	Colony Count (per 0.1 ml)	Frequency
R. leguminosarum 3894 (donor)	Kan_{50} Rif_{50} Tet_1	Clear	-
R. fredii HH303 (recipient)	Rif_{50} Kan_{50} Tet_1	25	4×10^{-9} (Background)
Transconjugants of *R. leguminosarum* *R. fredii*	Rif_{50} Kan_{50} Tet_1	145	2.3×10^{-8} (Transfer)

Table 2: Mean values of effect of inoculation with the different transconjugants on plant and nodule dry weights of soybean cultivars grown in greenhouse.

Cultivar	Plant dry weight g	Nodule dry weight mg/plant
Kent	1.235	297
Colombus	1.163	307
Crawford	1.071	403
Lee	0.925	198
$L.S.D._{0.05}$	0.075	25
$L.S.D._{0.01}$	0.114	38

Table 3: Mean values of plant and nodule dry weights on four soybean cultivars as a result of inoculation of soybean plants with individual transconjugants (greenhouse experiment).

Transconjugants	Plant dry weight*g g	Nodule dry weight/plant mg	% N in leaves
T_0 (control)	0.938	0	1.956
T_1	1.095	320	1.927
T_2	1.015	198	1.799
T_3	1.077	313	1.728
T_4	1.086	362	1.705
T_5	1.118	337	1.687
T_6	1.129	403	1.896
T_7	1.086	304	1.806
T_8	1.153	417	1.866
T_9	1.076	314	1.838
T_{10}	1.186	361	1.838
T_{11}	0.997	309	1.821
T_{12}	1.047	280	1.819
T_{13}	1.138	334	1.881
T_{14}	1.011	219	1.705
T_{15}	1.171	264	1.845
T_{17}	1.064	272	1.774
T_{18}	1.122	300	1.799
T_{19}	1.052	311	1.894
T_{20}	1.210	426	1.745
T_{22}	1.027	290	1.894
T_{23}	1.164	321	1.794
T_{27}	1.077	312	1.793
T_{28}	1.043	256	1.861
HH 303 (recipient)	1.236	257	1.401
$L.S.D_{0.05}$	0.139	54	1.820
$L.S.D_{0.01}$	0.184	71	NS

* Each value is the average of 16 replicates.

Table 4: Effect of inoculation of 4 soybean cultivars with the transconjugants on the nitrogen percentage in plant leaves (greenhouse experiment).

Transconjugants	Colombus*	Lee	Kent	Crawford
T_0 (control)	2.264	1.941	1.922	1.698
T_1	1.999	1.821	2.074	1.812
T_2	1.625	1.758	2.036	1.778
T_3	1.427	1.834	1.878	1.772
T_4	1.209	2.125	1.650	1.834
T_5	1.606	1.805	1.660	1.678
T_6	2.403	2.162	1.341	1.677
T_7	1.824	1.745	1.872	1.783
T_8	1.846	1.927	1.609	2.080
T_9	1.689	1.862	1.622	2.178
T_{10}	1.844	2.005	1.622	1.812
T_{11}	1.828	1.778	1.632	2.041
T_{12}	1.728	2.256	1.866	1.675
T_{13}	1.474	2.000	1.581	1.764
T_{14}	1.708	1.821	1.758	1.992
T_{15}	1.551	1.992	2.083	1.743
T_{17}	1.788	1.574	1.897	1.836
T_{18}	1.555	1.862	1.789	1.989
T_{19}	1.669	1.846	1.796	2.264
T_{20}	1.391	1.846	1.789	1.952
T_{22}	1.663	1.885	1.956	2.070
T_{23}	1.706	1.713	2.080	1.677
T_{27}	1.727	1.751	1.872	1.821
T_{28}	1.695	1.843	2.137	1.768
HH 303 (recipient)	1.745	1.957	1.903	1.810
Mean	1.719	1.884	1.817	1.860

*Each value is the average of four replicates. To compare difference between two means in column use the following values of L.S.D.

$L.S.D._{0.05} = 0.415$ $L.S.D._{0.01} = 0.546$

Nodule dry weight, however, exhibited a different pattern, while cultivar Lee had the lowest nodule dry weight, the highest nodule dry weight was found on Crawford.

The effects produced by the individual transconjugants on plant and nodule dry weights, averaged over all cultivars are shown in Table 3. Significant differences were observed in both plant and nodule dry weights imposed by the different transconjugants. Most of the transconjugants significantly increased plant dry weight over the uninoculated control, however, the highest plant dry weight was observed with the recipient strain HH 303.

As mentioned before, nodule dry weight was not correlated with plant dry weight. The recipient HH 303 strain exhibited a relatively low nodule dry weight although it produced the highest plant dry weight. Most transconjugants exhibited a higher nodule dry weight than the recipient. The highest nodule dry weight was noted for transconjugants T_{20}, LT_7 one T_5 with weight of 426, 417 and 403 mg/plant respectively.

Data in Table 4 summerize the effect of inoculation of the four soybean cultivars with the individual transconjugants on the N % in leaves of soybean plants. The highest N content was found in Colombus plants inoculated with T # 6, while inoculation with T # 12 produced the highest N % in Lee plants. Similarly, inoculation of Kent or Crawford with T # 28 and T # 19 gave the highest N % respectively. Values of N % in soybean could be arranged in the following descending order: Colombus 2.404; Crawford, 2.264; Lee 2.256 and Kent 2.137%.

Field experiments:

The influence of inoculation with the transconjugants on nodulation, plant growth, N % in leaves and seeds, pod and seed yield per plant and weight of 100 seed of the two cultivars; Clark and Crawford grown in 1988 is presented

Table 5: Effect of inoculation with the transconjugants on the nodule dry weight, plant dry weight, N % in leaves and seeds of both Clark and Crawford soybean cultivars grown in 1988 season (field experiment).

Trans-conjugants	Clark				Crawford			
	Nodule dry wt. mg/plant	Plant dry weight g/plant	% N in leaves	% N in seeds	Nodule dry wt. mg/plant	Plant dry weight g/plant	% N in leaves	% N in seeds
T_0 (Control)	000	10.55	4.10	5.84	35	16.70	4.04	5.84
T_1	660	12.25	3.28	5.31	170	12.33	4.09	5.73
T_3	552	12.40	3.66	6.20	377	14.90	3.74	6.27
T_4	140	13.00	3.65	5.62	380	12.08	3.60	5.64
T_5	573	15.93	3.49	5.36	190	10.98	3.56	5.43
T_8	188	9.70	3.37	5.24	270	12.73	5.97	6.08
T_9	328	12.28	3.56	5.47	477	11.90	3.60	5.64
T_{10}	550	11.66	3.34	5.79	208	20.33	4.08	5.92
T_{11}	387	13.63	3.56	5.77	358	11.43	3.19	5.52
T_{13}	423	13.53	3.95	5.94	575	16.23	3.75	5.48
T_{14}	348	13.13	3.31	5.71	330	11.08	3.64	5.54
T_{15}	350	11.68	3.54	5.59	190	10.60	3.48	5.68
T_{16}	193	12.03	3.30	5.75	583	14.88	4.36	6.12
T_{17}	138	13.88	3.59	6.09	150	16.75	4.12	6.51
T_{19}	428	9.58	3.59	5.66	178	12.05	3.50	5.73
T_{22}	400	12.83	3.35	5.86	488	11.53	3.35	5.36
T_{25}	227	11.75	3.68	6.09	300	13.80	3.76	6.26
T_{26}	493	11.50	3.88	5.79	220	8.70	3.87	5.93
T_{27}	148	19.00	4.17	6.12	178	12.50	4.34	5.76
T_{28}	242	11.08	3.76	5.53	300	16.18	3.87	6.26
HH 303	477	11.28	3.94	5.76	313	15.18	3.36	6.42
Mean	345	13.25	3.62	5.74	298	13.47	3.77	5.86
$L.S.D._{0.05}$	209	4.00	NS	0.53	209	4.00	NS	0.53
$L.S.D._{0.01}$	288	5.45	NS	0.70	288	5.45	NS	0.70

* Each value is the average of 4 replicates.

Table 6: Influence of inoculation with the transconjugants on yields of pods and seeds per plant and weight of 100 seeds of Clark or Crawford soybean cultivars grown in 1988 season (field experiment).

Trans-conjugants	Clark			Crawford		
	Pod wt. g/plant	Seed wt. g/plant	Wt. of 100-seeds g	Pod wt. g/plant	Seed wt. g/plant	Wt. of 100-seeds g
T_0 (control)	26.0	8.0	12.8	28.8	11.0	13.5
T_1	36.0	15.0	13.2	30.8	13.0	15.9
T_3	27.3	9.5	13.9	36.5	14.5	14.8
T_4	33.8	16.0	12.7	47.0	14.8	14.9
T_5	29.0	10.3	12.8	38.3	15.3	14.8
T_8	29.8	9.0	13.3	38.0	12.3	13.7
T_9	34.0	9.5	14.0	33.0	12.8	13.2
T_{10}	31.3	9.8	14.3	30.0	13.0	15.0
T_{11}	27.0	13.3	14.7	39.5	14.8	14.2
T_{13}	31.3	13.0	14.5	44.0	18.3	14.2
T_{14}	27.3	10.3	14.3	37.0	12.8	14.6
T_{15}	33.3	9.5	14.9	40.8	14.0	13.3
T_{16}	40.3	15.0	12.2	42.3	19.0	15.5
T_{17}	35.8	12.8	15.0	41.3	14.0	13.7
T_{19}	38.0	13.0	12.6	35.0	14.5	13.8
T_{22}	33.0	13.0	13.7	28.0	11.8	14.6
T_{25}	27.0	10.0	12.9	45.8	21.3	13.6
T_{26}	30.0	11.3	11.7	46.3	13.8	14.1
T_{27}	42.8	14.5	13.9	53.0	25.3	15.8
T_{28}	33.3	14.0	14.3	44.5	17.3	13.6
HH 303	32.0	9.8	15.2	31.5	9.5	12.9
Mean	32.3	13.7	13.7	38.6	14.9	14.3
$L.S.D._{0.05}$	N.S	4.4	N.S	N.S	4.4	N.S.
$L.S.D._{0.01}$	N.S	6.1	N.S	N.S	6.1	N.S

in Tables 5 and 6. In both cultivars, N % in leaves did not significantly affected by inoculation with the transconjugants (Table 5). Clark plants produced greater mean value of nodule dry weight than did Crawford plants. Inoculation of Clark and Crawford seeds with T_{17} yielded the lowest nodule dry weight; 138 and 150 mg/plant respectively. Pod yield/plant as well as weight of 100 seeds in either Clark or Crawford did not significantly affected by inoculation with the transconjugants during 1988 season (Table 6). However, inoculation with most transconjugants resulted in significant increases in seed yield of the two cultivars over the uninoculated control plants. Table 7 summerize the overall main effect of inoculation with the transconjugants on the same parameters tested previously on soybean cultivars. In general, most transconjugants caused significant increases in nodule dry weight in comparison with the uninoculated (T_0) plants. The following transconjugants T_1, T_3, $T_{13}$and T_{22} produced the highest nodule dry weight values in comparison with the recipient strain HH 303. On the other hand, inoculation with T_{17} and T_{27} gave the lowest values of nodule dry weights. N % in leaves, pod dry weight and weight of 100 seeds were not significantly affected by inoculation with the transconjugants. Contraversly, yield of seeds/plant was significantly different in inoculated plants. Some transconjugants did not significantly increased the seed yield in comparison with the control or the recipient. The highest seed yield was noted with T_{27} although it recorded the lowest nodule dry weight. Some transconjugants exhibited nonsignificant increases in seed yield comparatively with the uninoculated or the recipient strain HH 303. However, the transconjugants differed in their influences on the yield of soybean seeds.

The effect of inoculation with the transconjugants on nodulation, N % in leaves a seeds and yield of seeds and pod per plant and weight of 100 seeds of both Clark and Crawford plants in 1989 season is shown in Table 8, 9 & 10.

Table 7: The overall main effect of inoculation with the trans-conjugants on nodule dry weight, plant dry weight, N % in leaves and seeds, 100-seed weight and yields of pods and seed/plant grown in 1988 season (field experiment).

Trans-congujants	Nodule dry wt. mg/plant	Plant dry weight g/plant	% N in leaves	% N in seeds	Pod yield g/plant	Seed yield g/plant	Wt. of 100-seed
T_0 (control)	18	13.6	4.07	5.84	27.38	13.16	9.50
T_1	415	12.3	3.68	5.52	33.38	14.56	14.00
T_3	465	13.7	3.70	6.23	31.88	14.39	12.00
T_4	260	12.5	3.63	5.63	40.38	13.76	15.38
T_5	381	13.5	3.52	5.39	33.63	13.68	12.75
T_8	229	11.2	3.67	5.66	33.88	13.48	10.63
T_9	402	12.1	3.58	5.56	33.50	13.61	11.13
T_{10}	379	15.9	3.71	5.86	30.63	14.65	11.38
T_{11}	373	12.5	3.37	5.65	33.25	14.44	14.00
T_{13}	499	14.9	3.85	5.71	37.63	14.33	15.63
T_{14}	339	12.1	3.47	5.63	32.13	14.44	11.50
T_{15}	270	11.1	3.51	5.64	37.00	14.13	11.75
T_{16}	388	13.5	3.83	5.93	41.25	13.86	17.00
T_{17}	144	15.3	3.85	6.30	38.50	14.34	13.38
T_{19}	303	10.8	3.54	5.70	36.50	13.19	13.75
T_{22}	444	12.2	3.35	5.61	30.50	14.15	12.38
T_{25}	264	12.8	3.72	6.18	36.38	13.30	15.63
T_{26}	356	10.1	3.87	5.86	38.13	12.71	12.50
T_{27}	163	15.8	4.25	5.94	47.88	14.85	19.88
T_{28}	271	13.6	3.82	5.90	38.88	13.92	15.63
HH 303	395	13.2	3.65	6.10	31.50	14.08	9.63
Mean	321	12.99	3.69	5.80	35.44	12.92	13.31
L.S.D.$_{0.05}$	142	2.7	N.S	0.37	N.S	N.S	2.99
L.S.D.$_{0.01}$	178	3.6	N.S	0.49	N.S	N.S	3.96

Table 8: Effect of inoculation with the transconjugants on the nodule dry weight, plant dry weight, N % in leaves and seeds of both Clark and Crawford soybean cultivars grown in 1989 season (field experiment).

Trans-conjugants	Clark				Crawford			
	Nodule dry wt. mg/plant	Plant dry weight g/plant	% N in leaves	% N in seeds	Nodule dry wt. mg/plant	Plant dry weight g/plant	% N in leaves	% N in seeds
T_0 (Control)	203	18.4	2.08	5.68	170	23.1	2.31	5.76
T_1	468	28.5	2.72	6.11	470	21.8	2.96	5.99
T_3	600	21.2	2.82	5.93	810	24.0	2.73	5.93
T_4	658	25.0	2.82	6.23	840	27.6	3.14	5.98
T_5	743	32.5	3.02	6.08	950	21.7	2.63	5.93
T_8	557	29.9	2.69	5.94	858	25.6	2.66	5.84
T_9	420	31.1	2.58	6.13	652	25.6	2.75	5.88
T_{10}	752	27.6	2.79	6.11	413	21.6	2.35	6.08
T_{11}	738	30.7	2.89	6.16	932	28.1	2.62	5.86
T_{13}	468	29.8	3.19	5.85	613	31.6	2.75	6.04
T_{14}	622	23.3	2.81	6.11	843	28.3	2.73	5.78
T_{15}	432	23.6	3.00	5.67	357	28.0	2.24	5.99
T_{16}	495	29.3	2.77	6.24	720	32.3	2.47	5.61
T_{17}	670	31.2	3.01	6.31	517	22.7	2.65	6.28
T_{19}	692	26.3	2.79	6.06	622	23.6	2.60	5.70
T_{22}	247	24.2	2.48	6.08	177	28.5	2.74	5.98
T_{25}	500	29.3	2.94	5.97	492	24.7	2.66	5.85
T_{26}	387	22.2	2.98	6.31	923	21.9	3.08	6.19
T_{27}	370	19.1	2.72	6.22	442	22.3	2.75	5.81
T_{28}	608	20.4	2.71	6.12	995	31.3	2.52	6.12
HH 303	595	26.7	3.02	6.08	872	28.8	2.95	5.78
Mean	534	26.2	2.80	6.07	613	25.9	2.68	5.92
$L.S.D._{0.05}$	288	8.17	0.31	0.29	288	8.17	0.31	0.29
$L.S.D._{0.01}$	383	10.87	0.42	0.41	383	10.87	0.42	0.41

* Each value is the average of 4 replicates.

Table 9: Influence of inoculation with the transconjugants on yields of pods and seeds/plant and weight of 100 seeds of Clark and Crawford soybean cultivars grown in 1989 season (field experiment).

Trans-conjugants	Clark			Crawford		
	Pod wt. g/plant	Seed wt. g/plant	Wt. of 100-seeds g	Pod wt. g/plant	Seed wt. g/plant	Wt. of 100-seeds g
T_0 (control)	37.9	12.5	13.7	31.3	13.6	13.9
T_1	39.1	17.6	13.1	29.4	13.8	14.1
T_3	51.5	19.8	14.2	44.5	18.2	14.5
T_4	55.7	23.0	15.0	31.4	14.6	15.3
T_5	45.4	17.7	14.4	33.5	15.0	14.1
T_8	43.7	20.2	13.4	38.9	18.3	13.4
T_9	53.8	19.6	14.0	38.5	16.9	13.8
T_{10}	40.6	17.7	15.4	42.9	17.8	14.4
T_{11}	40.4	21.7	13.9	43.8	19.7	14.8
T_{13}	40.2	21.4	15.0	43.1	18.6	13.9
T_{14}	61.9	24.1	14.9	37.9	17.9	14.0
T_{15}	50.5	18.7	14.5	34.4	16.1	14.7
T_{16}	41.5	20.5	14.5	38.9	18.2	13.3
T_{17}	45.7	21.6	15.2	44.6	19.1	15.4
T_{19}	31.3	14.1	13.8	53.3	19.9	14.3
T_{22}	34.0	16.2	13.9	36.1	16.3	14.6
T_{25}	39.3	17.0	13.9	47.2	23.6	14.1
T_{26}	42.6	20.7	14.3	44.5	17.8	14.2
T_{27}	37.6	16.7	14.3	50.2	24.4	14.7
T_{28}	45.5	18.1	14.4	30.9	13.9	14.0
HH 303	30.5	15.4	15.5	39.1	16.7	14.1
Mean	43.3	18.8	15.2	39.7	17.6	14.3
$L.S.D._{0.05}$	11.9	5.5	N.S	11.9	5.5	N.S
$L.S.D._{0.01}$	16.9	7.8	N.S	16.9	7.8	N.S

Table 10: The overall effect of inoculat[illegible] he trans-conjugants on nodule dry weight, plant dry weight, N % in leaves and seeds, 100-seed weight and yield of pods and seeds/plant grown in 1989 season (field experiment).

Trans-congujants	Nodule dry wt. mg/plant	Plant dry weight g/plant	% N in leaves	% N in seeds	Pod yield g/plant	Seed yield g/plant	Wt. of 100-seeds
T_0 (control)	186	20.7	2.20	5.72	34.6	13.1	13.85
T_1	469	25.1	2.84	6.05	34.3	15.7	13.61
T_3	205	22.6	2.77	5.93	48.0	18.9	14.34
T_4	774	26.3	2.98	6.10	43.6	18.8	15.15
T_5	846	27.1	2.83	6.01	39.4	16.3	14.26
T_8	707	27.8	2.68	5.89	41.3	19.3	13.36
T_9	536	28.3	2.67	6.01	46.1	18.2	13.91
T_{10}	582	24.6	2.57	6.10	41.7	17.7	14.88
T_{11}	835	29.4	2.75	6.01	42.1	20.7	14.35
T_{13}	540	30.7	2.98	5.95	41.6	19.9	14.49
T_{14}	732	25.8	2.77	5.94	49.9	20.9	14.43
T_{15}	395	25.8	2.62	5.83	42.4	17.4	14.59
T_{16}	608	30.8	2.62	5.92	40.2	19.4	13.90
T_{17}	594	26.9	2.83	6.30	45.1	20.4	14.05
T_{19}	877	24.9	2.70	5.88	42.3	17.0	14.26
T_{22}	710	26.3	2.61	6.23	35.1	16.3	14.26
T_{25}	496	27.0	2.80	5.91	43.3	20.3	14.00
T_{26}	655	22.0	3.03	6.25	43.5	19.2	14.24
T_{27}	406	20.7	2.73	6.02	43.9	20.5	14.50
T_{28}	801	25.8	2.62	6.12	38.2	16.0	14.21
HH 303	734	27.7	2.98	5.93	34.8	16.0	14.78
Mean	604	26.0	2.74	6.00	41.5	18.2	14.26
L.S.D.$_{0.05}$	202	5.76	0.22	0.20	7.7	3.7	0.49
L.S.D.$_{0.01}$	267	7.62	0.29	0.27	10.2	4.8	1.32

Data indicate that Crawford plants gave higher significant nodule dry weight than did Clark (Table 8). N % in leaves or seeds of Clark was greater than that of Crawford. Inoculation with T_3, T_4, T_5, T_8, T_{10}, T_{11}, T_{14}, T_{16}, T_{17}, T_{19}, T_{25} and T_{28} had significant influence on nodule dry weight and N % in leaves or seeds of Clark plants compured with the uninoculated control plants. However, none of these transconjugants significantly improved nodulation, plant growth or seed yield of Clark plants over those inoculated with the recipient strain HH 303 (Table 9).

The overall influence of inoculation with the transconjugants on all parameters tested on the two soybean cultivars is shown in Table 10. Data show that inoculation with T_{19}, T_5, T_{11} and T_{28} resulted in the highest values of nodule dry weights comparatively with the other transconjugants. However, inoculation with the transconjugants did significantly increase nodule dry weight over the inoculation with the recipient strain HH 303. Generally, transconjugants caused significant increases in plant dry weight compared with the control plants. Similar trend could be observed on N % in leaves. Pod and seed yield/plant were significantly affected by inoculation with the transconjugants.

A quick glance at results of the field experiments of 1988 and 1989 seasons reveals that nodulation of soybean plants by the transconjugants was better in 1989 season. The same trend goes on the plant dry weight. Leaves of soybean plants grown in 1989 season had less N content than did plants of 1988 season. N % in seeds did not differ much in plants grown during the two seasons. Plant dry weights were correlated with yields of pods and seeds/plant. Again, in 1989 more dry plant materials were produced than did in 1988. This led to the fact that Clark plants outyielded Crawford plants in pod yield and consequencely seed yield/plant in 1989 season. The overall effect of inoculation with the transconjugants followed the same way regards all the parameters tested.

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