African Journal of Plant Science Vol. 5 (6), pp. 337-348, June 2011
Available online at http://www.academicjournals.org/ajps
ISSN 1996-0824 ©2011 Academic Journals

Full Length Research Paper

A study of intraspecific hybrid lines derived from the reciprocal crosses between wild accessions and cultivated cowpeas (*Vigna unguiculata* (L.) Walp.)

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Accepted 28 April, 2011

Cowpea (*Vigna unguiculata* (L.) Walp.) is an essential food legume in the (sub) tropical areas. Reciprocal crosses were performed using wild accessions for transferring genes of interest to cultivars (524-B/IT84S-2049). Of these, a low number of seeds were obtained because of crossing barriers. A seed sample of 17 F_1 hybrids germinated after germination was 69.09%. A subdivision into seed sterility, partial seed fertility and seed fertility was observed. Seed sterility was possibly due to chromosomal disturbances that occur in endosperms and embryos during early seed development. In partial seed fertility, vigorous plants flowered about 128 DAE but no pods were formed because of floral abscission at anthesis. In plants of (524-B (♀) × *tenuis* (♀)) combination, partial sterility was caused by incomplete male sterility. In 13 F_1 hybrids, viability among populations was reduced (67.25%). Eleven F_1 hybrids were grown in the greenhouse to produce F_2 seeds by natural self-pollination. Plants were characterised through using cowpea descriptors. Variability, in terms of morphological characters in adult F_1 plants, was established. Differences in vegetative, inflorescence and fruit characters were described after full plant development. The results show that the adult F_1 plants appear to be dominant for wild vegetative and inflorescence characters expressed by one or two of the parents in bi-parental and reciprocal crosses, for exception plants derived from crosses in which *alba* wild forms served as male parent showing the same morphological characteristics as the cultivated female parents, as evidenced by the traits inherited from cultivars. The heterotic status exhibited by emerged plants, supposes that the latter types inbred at the first generation confirm that the wild parents involved in these hybrids represent a wide diversity of germplasm. Seed characteristic studied in F_2 has not shown segregation because of recessive type absence, suggesting that morphological traits should be monitored.

Key words: Reproductive barriers, seed fertility, introgressive hybridisation, wild character inheritance.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) (2n = 22) is an essential grain legume crops in tropical and subtropical regions (Rachie and Roberts, 1974). It provides inexpensive proteins and can be prepared easily in a number of edible forms, such as tender green shoots and leaves, immature pods, and green and dry seeds (Fery, 1980; Ehlers and Hall, 1997).

In small-scale agriculture, the major problem is the extremely severe damage (up to 90%) caused by

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post-flowering insect pests in cowpea, including the legume pod borer *Maruca testulalis* Geyer and the complex of pod-sucking bugs, including C*lavigralla tomentosicollis* Sthal, *Anoplecnemis curvipes* F. and *Riptortus dentipes*F. (Ehlers and Hall, 1997; Olufajo and Singh, 2003). Wild taxa of *V. unguiculata* identified within the primary gene pool are known as a potential source of resistance to major insect pests (Padulosi and Ng, 1990).

Reciprocal crosses were performed between wild accessions and cultivated cowpeas for transferring post-flowering insect pest resistance to cultivars (524-B/IT84S-2049). According to the total number of 487 flowers that were pollinated during the hybridisation programme in 1999, compatible cross number (that is, mature pod number/total number of reciprocal crosses) produced 78 (16.01%) mature pods in 20 to 25 days after pollination (Lelou and Van Damme, 2006). The number of incompatible crosses was about 391 (80.7%), revealing the presence of reproductive barriers after fertilisation/seed development, as reported by Barone and Ng (1990); Lelou and Van Damme (2006).

Viability is assayed using the tests of germination (Scott et al., 1998). These present attempts were conducted to estimate seed fertility yielded in F_1 hybrid lines derived from intraspecific reciprocal crosses between wild accessions x cultivars in order to identify some hybrid lines with excellent response after the experimental tests in 2000/2001 crop seasons.

The incorporation of genes of one species into the gene pool of another is termed introgressive hybridisation (Yoshinari, 2007). To illustrate multi-step processes by which genetic materials from wild accessions infiltrate into cowpea cultivars and vice-versa, the characterisation in each of F_1 cowpea adult-hybrids was conducted in order to examine the extension of variability range after different morphological character inheritance.

The former characters are primitive and the latter are derived types. During hybridisation programme, the derived types are expressed in these hybrids and in order to make favourable genetic material.

Thus three morphological types (vegetative, inflorescence and fruit characters) were characterised amongst F_1 cowpea hybrid lines. From a number of the F_1 cowpea hybrid lines that emerged, the expression of characters was variable, indicating that environmental conditions as well as the segregation of minor genes influence penetrance. It is known that characters are not phenotypically identical. It is possible e.g. that leaf texture type can be possessed less advantage in cultivation under greenhouse conditions than it apparently possesses in nature.

The objective of this experimental study was, with regard to post-zygotic barriers that build up after successful fertilisation, to determine whether the expression of different genes derived from cultivars and wild accessions grown in the greenhouse could provide an excellent opportunity to study evolutionary processes in all F_1 hybrid populations. On the basis of these results, it can be expected to select new cultivars of cowpea by selection for plant resistant to insect-pest flowering and adaptations to variable environments over several generations.

MATERIALS AND METHODS

Hybrid seed materials

The seed samples of the original F_1 cowpea hybrid lines obtained during a hybridisation programme were executed in a tropical greenhouse between wild accessions and cultivated cowpeas during the winter season of 1999 at the Laboratory of Tropical and Subtropical Agriculture and Ethnobotany, Faculty of Bioscience and Engineering (Ghent University, Belgium). In Table 1, we characterised wild and cultivated parental accessions of cowpea manipulated in this breeding programme.

Seed germination experiments

The seed samples of 17 F_1 hybrid lines of cowpea were subjected to laboratory germination tests, as described below during the spring season of 2000 to 2001. In Table 2, the different 17 reciprocal F_1 hybrid combinations of cowpea, pollination number/hybrid combination, aborted ovule number and their pod and seed production, are reported respectively. These seed samples collected in all F_1 hybrid lines of cowpea exhibited seed coat dormancy, as wild parental accessions of cowpea.

The methods of seed preparation and sowing were an adaptation of the techniques described by Lelou (1997). Physical dormancy is caused by water impermeable layers of palisade cells in the seed or fruit coat which control water movement. Prior to the conduction of the germination tests, these seeds were treated to break physical dormancy. Seeds were surface-sterilised in 70% ethanol for one minute and disinfected by $(CLO_3)_2Ca$ (5%) for five minutes, rinsed three times in distilled water. To induce a better permeability, seed coats were mechanically scarified mildly with a scalpel in order to overcome dormancy during germination. To prevent contamination by micro-organisms, these above mentioned seeds treated were immersed for a minute in the fungicide Thiram ($C_6H_{12}N_2S_4$).

After treatment, all seed samples were sown and randomly dispersed across moistened filter paper into Petri dishes. Water was introduced in sufficient and enough quantities into the closed Petri dishes and kept in a 'Philips' incubator: 24°C day and 20°C night temperatures at 12 h photoperiod/day. The results of germination test were recorded daily for 7 days during laboratory experiments.

Seedling emergence

The durations from sowing to emergence of the radicle through the seed coat of seeds treated, were observed among seed samples constituting the cowpea F_1 hybrid lines. Petri dishes placed into an incubator were checked daily in order to detect: a) radicle elongation, that is, observation of radicle visible through seed testa, when it exceeded 4 or 5 mm out extended cotyledons; and b) seedlings destroyed, that is, seeds ungerminated in which seedlings were regarded to have died when it feel over a most of the seedling turned yellowish or brownish.

Once radicle definitively emerges, seedling is subsequently potted in small plastic pots (diameter 5 cm) and placed into the growth chamber with same climatic characteristics that the incubator.

At the two leave-stages, the young plantlets belonging to adult F_1 hybrids of cowpea were individually grown in plastic pots of

Table 1. Origin, biological status and some morphological and pollen male fertility characteristics of parental genotypes of cowpea (*Vigna unguiculata* (L.) Walp.) used in our breeding programme conducted under greenhouse at Lab. of tropical agriculture in Faculty of Bioscience (Ghent University, Belgium) in 1999/2000/2001.

Parental genotypes and accession number	Biological status	Biological cycle	Country of origin	Growth habit	Plant hairiness	Leaf texture	Terminal leaflet shape	Leaf marking	Flower colour	Pod colour	Testa texture	Plant number/ genotype	Pollen fertility (%)
subsp. *burundiensis* NI 456	Wild	Allogamous	Burundi	Indeterminate and prostrate	Glaberscent	Cariaceous	Sub-globose	Absence	Violet	Pale tan	Rough	8	79.0
subsp. *baoulensis* NI 749	Wild	Allogamous	Cameroon	Indeterminate and prostrate	Glaberscent	Cariaceous	Sub-globose	Absence	Violet	Pale Tan	Rough	8	83.5
subsp. *pawekiae* MT 53	Wild	Allogamous	Malawi	Indeterminate and prostrate	Glaberscent	Cariaceous	Sub-globose	Absence	Violet	Dark brown	Smooth to rough	8	86.0
subsp. *letouzeyi* NI 1420	Wild	Allogamous	Cameroon	Indeterminate and prostrate	Glaberscent	Cariaceous	Sub-globose	Absence	Violet	Pale tan	Rough	8	84.5
subsp. *alba* SP 145	wild	Allo-autogamous	Congo	Indeterminate and prostrate	Glaberscent	Cariaceous	Sub-globose	Presence	Violet	Pale tan	Rough	8	88.0
subsp. *tenuis* sp 167	wild	Allo-autogamous	South Africa	Indeterminate and prostrate	Glaberscent	Cariaceous	Hastate	Presence	Violet	Pale tan	Rough	8	92.5
subsp. *pubescens* NI 979	wild	Allo-autogamous	Kenya	Indeterminate and prostrate	Pubescent hairs	Cariaceous	Sub-hastate	Presence	Violet	Pale tan	Rough to wrinkled	8	92.5
var. *spontanea* NI 945	weed	Autogamous	Niger	Indeterminate and prostrate	Glaberscent	Cariaceous	Sub-globose	Presence	Violet	Pale tan	Smooth to rough	8	78.0
var. *unguiculata* 524-B	Cultivar	Autogamous	USA	Indeterminate and spreading	Glaberscent	Membranous	Globose	Absence	White	Dark tan	Smooth	4	87.0
var. *unguiculata* IT84S-2049	Cultivar	Autogamous	USA	Indeterminate and spreading	Glaberscent	Membranous	Globose	Absence	White	Dark tan	Smooth	3	87.0

diameter 20 cm containing the same standard potting soil in the greenhouse, in order to produce the F_2 generation by the natural self-fertilisation.

Data measurement

The tests of germination were immediately carried out after incubation. The samples composed of 110 seeds considered as well-developed in moistened derived from 17 hybrid combinations variant from 4 to 10 seeds/hybrid linewere manipulated, according to ISTA procedures (1976).

Variability of seed number tested was due to the difference yielded in seed samples. The percentage of germination was calculated by use of the following formula:

$$[\Sigma_i^{\ n} n_i / \Sigma_I^{\ n} N_i] \times 100 \qquad (1)$$

where: n_i is the number of germinated seeds; and N_i is the total number of seed placed for germination.

Pollen fertility was assessed by staining fresh pollen grains with aceto-carmine, according to Belling (1921), and calculating the percentage of deeply stained pollen grains for hybrid line. The sample for determining the pollen fertility was approximately 200 pollen grains from 2 flowers/adult hybrid plant. The pollen viability image was captured and counted with a 'Zeiss Axioskop' optical microscope (10 × objective). Pollen measurement was made on the F_1 plants. Pollen size measurement was subjected through using the ocular scale on the microscope, according to microscopic observation techniques described

Table 2. Distribution of F_1 materials obtained, according to the different 17 hybrid combinations derived from reciprocal crosses between wild accessions and cultivated cowpea (*Vigna unguiculata (L.) Walp.*).

Hybrid combinations ♀ parents × ♂ parents	Pollinated flower number/hybrid combination	Aborted ovule number/hybrid combination	Harvested mature pod number/hybrid combination	Yield of harvested seed hybrid number/hybrid combination
524-B × *pawekaie*	18	16	1	15
pawekiae × 524-B	29	26	2	6
524-B × *alba*	35	30	5	26
alba × 524-B	19	14	3	15
524-B ×*tenuis*	20	18	2	4
524-B × *pubescens*	7	2	5	27
524-B × *spontanea*	3	1	1	5
IT84S-2049 × *baoulensis*	21	19	2	22
IT84S-2049 × *pawekiae*	12	7	5	63
pawekiae × IT84S-2049	30	20	10	53
IT84S-2049 × *alba*	47	36	10	38
alba × IT84S-2049	33	23	7	75
IT84S-2049 ×*tenuis*	21	16	4	14
IT84S-2049 × *pubescens*	33	22	11	73
pubescens × IT84S-2049	18	13	11	73
IT84S-2049 × *spontanea*	23	22	1	8
spontanea × IT84S-2049	12	7	5	42

by Rapilly (1968).

Phenotypic expression of morphological traits

To have an idea whether wild gene incorporation into cultivated forms of *V. unguiculata* was yielded after some artificial hybridisations in the F_1 generation, the stable adult plants derived from reciprocal crosses between wild accessions and cultivated cowpeas were examined. An experimental study was conducted in the greenhouse. Nine morphological characters in which five vegetative, two inflorescence and two fruit characters were characterised, according to the descriptors for cowpeas. The cowpea F_1 hybrid line constituting the sample-adult plant was composed of 3 plants, with exception for 3 F_1 hybrid lines derived from (524-B (♀) × *tenuis* (♂) and 524-B (♀) × *spontanea* (♂) combinations in which 2 plants were formed and in IT84S-2049 (♀) × *baoulensis* (♂) combination 1 plant was obtained. Fourteen hybrid combinations constituting the biodiversity at the first generation susceptible to inherit the latter types were examinedduring vegetative and reproductive stages.

Biodiversity range description

Distribution of the F_1 adult hybrids, five qualitative and morphological, two inflorescence and two fruit characters as following as: growth habit, plant hairiness, leaf texture, terminal leaflet shape, leaf marking, flower colour, gametophytic male, pod colour and texture of testa seed characters were studied. Amongst these selected characters, the frequencies of distribution were used to determine for each continuously varying traits with exception for pod colour and texture of testa seed characters in which 36 F_1 adult plants were controlled

Growth habit

This character was visually evaluated, 6 weeks after planting, taking into consideration in the indeterminate spreading traits of hybrid plants. The scale used was:

(i) Indeterminate spreading and not climbing, that is, lower branches touch the ground.
(ii) Indeterminate spreading and prostrate i.e. plants flat on ground; branches spread several metres.
(iii) Indeterminate spreading and climbing.
(iv) Indeterminate spreading and climbing

It was observed that the adult vigour plants in hybrid accessions lines tended to have an indeterminate conformation. Distribution of the hybrid accession lines over the three-growth habit groups was respectively 78.58 and 21.42% for Groups 2 and 3.

Plant hairiness

According to descriptors for cowpeas, stems, leaves and pods were either glabrescent, that is, lacking hairs or tendency to become hairless vs. pubescent short hairs. These both characteristics were assigned the Indexes 3 and 5, respectively. From 14 hybrid accession lines observed, only 1 hybrid line (3 sample-adult plants/F_1 hybrid line of cowpea) was assigned the Index 5, that is, pubescent and short hairs. In other words, almost 92.59% of hybrid accessions had glabrescent stems, leaves and pods.

Leaf texture

Leaf texture was visually observed 6 weeks after planting, according to the following scale:

(i) Cariaceous
(ii) Intermediate
(iii) Membranous

The distribution of the hybrid accession lines over the three groups of leaf texture was respectively 78.58 and 21.42% for Groups 1 and 3.

Terminal leaflet shape

A visual evaluating of the terminal leaflet shape took place 6th week after planting, according to the following scale:

(i) Globose (having shape as a globe)
(ii) Sub-globose
(iii) Sub-hastate
(iv) Hastate (triangular, shaped like a spear point)

The distribution of the hybrid accessions over the four groups of terminal leaflet shape was 71.42, 21.42 and 7.14%, for Groups 2, 1 and 3, respectively.

Leaf marking

Observations were done for this character for 6 weeks after planting and the presence vs. absence of V mark was observed on leaflets: 71.42% of samples are free of leaf marking and 28.57% are leaf marked.

Flower colour

Flower pigmentation was visually evaluated using the following scale:

(i) White
(ii) Violet
(iii) Mauve-pink
(iv) Other

About 38 sample-adult hybrid plants were inspected from 45 to 150 days with the first flowers appearing 1 week earlier. The distribution of the hybrid accessions over the four groups of flower colour was 14.28 and 85.72% for Groups 1 and 2, respectively.

Gametophytic male

The results of pollen fertility from 14 cowpea F_1 hybrid lines were presented in range of 60, 74 and 86.5% for Groups 1, 2 and 3 respectively.

Pod colour

A visual rating of pod colour took place after harvesting and drying, according the following scale:

(i) Pale tan or straw
(ii) Dark tan
(iii) Dark brown
(iv) Black or dark purple
(v) Other

The number of hybrid accessions was reduced to 36 hybrid plants. Because of 2 adult plants form (524-B (♀) × *tenuis* (♂)) combinations flowered and collapsed after emergence. Distribution frequency of hybrid accessions over the five groups of pod colour was respectively 46.15, 38.46 and 15.38% for Groups 1, 2 and 3.

Testa seed texture

Nine textures of testa seed were identified in the collection. These textures presenting in the cowpea descriptors are:

(i) 1 – smooth
(ii) 3 – smooth to rough
(iii) 5 – rough (fine reticulation)
(iv) 7 – rough to wrinkled
(v) 9 – wrinkled (coarse folds on the testa)

Distribution frequency for texture of testa concerned notably 13 hybrid accessions, shown for F_2 seeds in 16.66, 33.33, 41.66 and 8.33% for Groups 1, 3, 5 and 7, respectively.

EXPERIMENTAL RESULTS AND DISCUSSION

Seed germination evaluation

According to the texture of seed testa derived from genetic materials of the reciprocal crosses between wild accessions × cowpea cultivars, it was provided evidence that all hybrid seeds inherited wild female parent characteristics at the first generation. The texture of seed testa was phenotypically identical, according to the origin of wild parental strains. Genetically, the effects expressed in these F_1 seeds allowed to show that the success of germination test depends in part on wild parent material source. From these data reported, the germination of dominant seed coat dormancy identified amongst 17 cowpea intraspecific F_1 hybrids obtained is summarised in Table 3.

Seeds germinated

There was a wide variation in fertility level in the seeds, in accordance with the cross-results. The rate of germinated

Table 3. Results of germination tests, of F_1 seeds derived from reciprocal crosses between wild accessions and cultivated cowpeas (*Vigna unguiculata* (L.) Walp.), according to ISTA procedure (1976), germination was conducted in Petri dishes containing a moistened filter paper and placed in a Philips incubator with specific characteristics (day and night temperatures (24/20°C), fluorescent illumination system (black and light, 365 nm, 4 watt) and photoperiod/day (12 h)

Hybrid combinations ♀ parents x ♂ parents	Total number of F_1 seeds sown / hybrid combination	Number of germinated seeds at the beginning and final periods of the tests		Total number of seeds germinated after 7 days	Germinated seeds F_1 percent/hybrid combination
-	-	At the beginning period of the test	At the final period of the test	-	-
524-B × *pawekiae*	6	2	4	6	100
pawekiae × 524-B	6	3	3	6	100
524-B × *alba*	6	2	4	6	100
alba × 524-B	6	1	5	6	100
524-B × *tenuis*	4	0	2	2	50
524-B × *pubescens*	7	0	0	0	00
524-B × *spontanea*	5	0	2	2	40
IT84S-2049 × *baoulensis*	8	0	1	1	12.5
IT84S-2049 × *pawekiae*	6	2	4	6	100
pawekiae × IT84S-2049	8	2	6	8	100
IT84S-2049 × *alba*	7	3	4	7	100
alba × IT84S-2049	7	2	5	7	100
IT84S-2049 × *tenuis*	6	0	0	0	00
IT84S-2049 × *pubescens*	9	0	0	0	00
pubescens × IT84S-2049	5	2	3	5	100
IT84S-2049 × *spontanea*	4	2	2	4	100
spontanea × IT84S-2049	10	2	8	10	100
-	110 seeds	23 seeds	53 seeds	76 seeds	(mean) X = 69.09%

seeds after the test was 69.09 germinated percentage. The highest percent of germination (100%) was distinguished in 11 hybrid lines derived from crosses. Therefore, the seeds from (524-B (♀) × *tenuis* (♂)) combinations were second (50%) and these from (524-B (♀) × *spontanea* (♂)) combinations were third (40%). Finally, the percentage of germinated seeds of (IT84S-2049 (♀) × *baoulensis* (♂)) combinations was 12.5%. In this later case, the final counts made after 7 days showed that germination speed was absolute minimum. The results revealed that one seed germinated. However, the seedling was considered as normal in Petri dish.

Seeds ungerminated

The number of seeds ungerminated (30.91%) was determined after seedling counts. Final counts made after 7 days showed that any radicle elongation was observed or very short and turned yellowish in the following hybrid lines derived from these combinations: 524-B (♀) x *pubescens* (♂), IT84S-2049 (♀) × *tenuis* (♂) and IT84S-2049 (♀) × *pubescens* (♂).

Since the results of the standard of germination test showed defects as several factors such as abnormal embryo development, physiological immature at the harvested period, seed quality and yield are directly influenced by the genetic composition of seeds (Scott et al., 1998). Manz et al. (2005) reported that the relationships between germination test and radicle emergence initiation 24 h after imbibition and finished germination before 96 h are excellent, when artificial conditions provide favourable effect for viable seeds to germinate and to perform seedling growth.

The F_1 generation observation

Hybrid viability

Luo et al. (2005) reported that an individual is viable if it can survive the adult stage. Sahai (2000) indicated that the late stage embryo mortality is the main case of seed sterility. In these cases of sterility evidenced, the embryological disturbances should be caused by genetic factors (Rieseberg, 2001). In other circumstances, the adverse climatic effects can probably disturb the normal embryo development which varies considerably in different localities, localities, trees and species (Robertson *et al.,* 1996; Hall, 2003).

Adverse climatic effects can cause injury in any (early or late) stage of embryo development. Embryo survival depends on the severity of the injury (Sahai, 2000). According to Walsh et al. (2003), viability testing through germination is essential for the maintenance of a seed gene bank collection and can be a rapid way of identifying problems with the seed storage condition.

After sterility extraction among F_1 seeds derived from reciprocal crosses between wild accessions and cultivated cowpeas, the development of the next generation was required. Fertility indicates chromosome pairing between wild accessions × cowpea cultivars. However, the taxonomic studies in cowpeas revealed the close phylogenetic relationships between cowpea parent materials (Maréchal et al., 1978; Pasquet, 1993; Coulibaly et al., 2001).

The particular (524-B (♀) × *tenuis* (♂)) combination was studied; hence it was discovered in progeny line that vigorous plants flowered about 128 DAE (that is, days after emergence) (data no shown). This mutant was also characterised by failure of a majority of the flowers to develop beyond the bud stage with the result that it is nearly completely sterile. It is evidence that hybrid viability at the F_1 generation was found to be reduced (67.25%).

F_1 cowpea hybrid line characterisation

According to IBPGR (1982), the characterisation consists of recording those characteristics highly heritable that can be easily observed by eyes and expressed in all environments. These are usually represented by the morphological characters of plants (Table 4).

Hybrids of indeterminate spreading and prostrate × indeterminate spreading and climbing grown: A total of 38 hybrid accessions derived from between indeterminate spreading and prostrate × indeterminate spreading and climbing parents was studied. The F_1 was indeterminate spreading and prostate in grown habit. With regard to comparative parental characteristics; data analysis revealed that two lines derived (IT84S-2049 (♀) × *alba* (♂) and 524-B (♀) × *alba* (♂)) had produced the same morphological and characteristics as their respective cultivated female parents. We also observed white flower colour after the propagation of reproductive organ. These confirmed the presence of two infertile or apomictic lines among cowpea F_1 hybrid combinations.

Hybrids of pubescent short hairs × glabrescent: From 32 F_1 hybrid accessions examined, all hybrid lines were almost glabrescent stems, leaves and pods. In certain hybrids and particularly under greenhouse conditions the expression of the pubescence short hairs was observed in 3 hybrid lines derived from (*pubescens* (♀)×IT84S-2049 (♂)) combination.

Hybrids of cariaceous × membranous of leaf texture: Two different classes were studied at the first generation. One of the membranous parents was observed in these observed apomictic lines. Other F_1 hybrids indeterminate and prostrate inherited the cariaceous characteristic as the wild material parents. The distinction between types was clear and no difficulties were encountered during identification stage.

Hybrids of sub-globose and sub-hastate × globose of terminal leaflet shape: The particular sub-hastate character was discovered in allo-autogamous and perennial wild parental strain, that is, subsp. *pubescens*. The F_1 hybrids derived from (*pubescens* (♀) ≤ IT84S-2049 (♂)) combinations were completely inherited sub-hastate characteristics. A typical leaf trait was shown in Figure 1. In the F_1 hybrids formed with allogamous and other allo-autogamous perennial wild parents, the type of sub-globose terminal leaflet shape was generated. On this other hand, the type of globose terminal leaflet shape characteristic was observed among apomictic lines.

Hybrids of V mark on leaflets: All combinations derived from allo-autogamous perennial wild parents × cultivated parents, the F_1 hybrids shown V mark on leaflets. In combinations with allogamous perennial and annual wild parents, the leaf surfaces were leaf marking free.

Hybrids of yellow × purple flower colour: Flower colour of the F_1 plants was completely purple in F_1 hybrid lines. In fact, several researches published that in F_1 plant flower colour both genes control this trait. According to Fery (1980) white and purple flower are codominant at the first generation.

Hybrids of high × low percent pollen fertility: Pollen fertility in F_1 plants was determined by staining. The data from male fertility parental genotypes was high over one

Table 4. Data on morphological character characterisation carried out among 14 hybrid combinations derived from reciprocal crosses between wild accessions and cultivated cowpeas (*Vigna unguiculata* (L.) Walp.), according to descriptors for cowpeas.

Characters studied	Growth habit	Plant hairiness	Leaf texture	Terminal leaflet shape	Leaf marking	Flower colour	Pod colour	Testa texture	Pollen fertility percent	
-	Index	Index	Index	Index	Index	Index	Index	Index	> 70%	< 70%
Parental genotypes										
Wild accessions										
pawekiae	2	5	1	2	-	2	3	3	86.0	-
baoulensis	2	5	1	2	-	2	1	5	83.5	-
alba	2	5	1	2	+	2	1	5	88.0	-
tenuis	2	5	1	4	+	2	1	5	92.5	-
pubescens	2	3	1	3	+	2	1	7	86.0	-
spontanea	2	5	1	2	+	2	1	5	78.0	-
Cultivars										
524-B	3	5	3	1	-	1	2	1	87.0	-
IT84S-2049	3	5	3	1	-	1	2	1	78.0	-
F_1 hybrid combinations										
524-B × *pawekiae*	2	5	1	2	-	2	3	3	71.5	-
pawekiae × 524-B	2	5	1	2	-	2	3	3	72.5	-
524-B × *alba*	3	5	3	1	-	1	1	1	81.6	-
alba × 524-B	2	5	1	2	+	2	1	5	-	59.4
524-B × *tenuis*	3	5	3	1	-	2	-	-	70.0	-
524-B × *spontanea*	2	5	1	2	+	2	1	5	76.4	-
IT84S-2049 × *baoulensis*	2	5	1	2	-	2	1	5	72.7	-
IT84S-2049 × *pawekiae*	2	5	1	2	-	2	3	3	80.8	-
pawekiae × IT84S-2049	2	5	1	2	-	2	3	3	73.8	-
IT84S-2049 × *alba*	3	5	3	1	+	1	3	1	80.8	-
alba x IT84S-2049	2	5	1	2	+	2	2	5	72.7	-
pubescens × IT84S-2049	2	3	1	3	+	2	1	7	-	67.3
IT84S-2049 × *spontanea*	2	5	1	2	+	2	1	5	72.3	-
spontanea × IT84S-2049	2	5	1	2	+	2	1	5	84.3	-

Growth habit, Index 2: indeterminate and prostrate; Index 3: indeterminate spreading and climbing. Plant hairiness, Index 3: glabrescent; Index 5: pubescent and short appressed hairs. Leaf texture, Index 1: cariaceous; Index 3: membranous. Terminal leaflet shape, Index 1: globose; Index 2: sub-globose; Index 3: sub-hastate; Index 4: hastate. Leaf marking, index (-): absence; index (+): presence. Flower colour, Index 1: pale tan; Index 2: dark tan; index 3: dark brown.Testa texture, Index 1: smooth; Index 3: smooth to rough; Index 5: rough; Index 7: rough to wrinkle. Pollen fertility: percentage > 70 and < 70%

over one; however, low pollen fertility percentage was observed in plants from infertile line as (*alba* (♀) ×524-B (♂)) combination and in F_1 hybrid line of (*pubescens* (♀) × IT84S-2049 (♂)) combination. High pollen fertility was identified in plants derived from two infertile lines of (524-B (♀)× *alba* (♂)) and (IT84S-2049 (♀) × *alba* (♂)) combinations, in F_1 hybrid lines from (IT84S-2049 (♀)σ *pawekiae* (♂)) and (*spontanea* (♀) ×IT84S-2049 (♂))

Figure 1.The F_1 (*pubescens* (♀) × IT84S-2049 (♂)) hybrid line presenting the sub-hastate terminal leaflet shape and V marks on leaflet characteristics.

combinations.

Hybrids of dark tan and brown × straw pod colour: Pod colour derived from F_1 hybrids at the first generation presented the same phenotypic configuration as female parents in crosses there was maternal dominance inheritance occurred in all of hybrids (Birky, 1995).

Hybrids of rough × smooth seed testa texture segregated: In all of hybrids the F_1 textures of seed testa inherited seed characteristic of wild parental strains. After F_1 adult-plant development, all morphological characteristics belong to the first generation but seeds provided by these hybrids plants constitute the F_2 genetic material.

In the first generation character recombination amongst all hybrid individuals revealed that wild characters expressed by one or two of the parent in bi-parental and reciprocal crosses were dominant, except in pod colour in which maternal dominant effect encountered, has shown maternal dominance inheritance, according to Aliboth's et al. (1997) works, wild character dominance in the fertile hybrid lines derived from reciprocal crosses between wild accessions and cultivated cowpeas showed a heterotic status for morphological, inflorescence and fruit traits.

In the F_2 generation, 16.66 smooth, 33.33 smooth to rough, 41.66 rough and 8.33 rough to wrinkled types occurred in a ratio of 2 smooth: 2 rough individuals. The deficiency in the recessive class in F_2 may indicate lower viability of the mutant type. In seed testa texture character in which individuals were distinguished in 2 classes, the F_2 ratio observed may indicate segregation

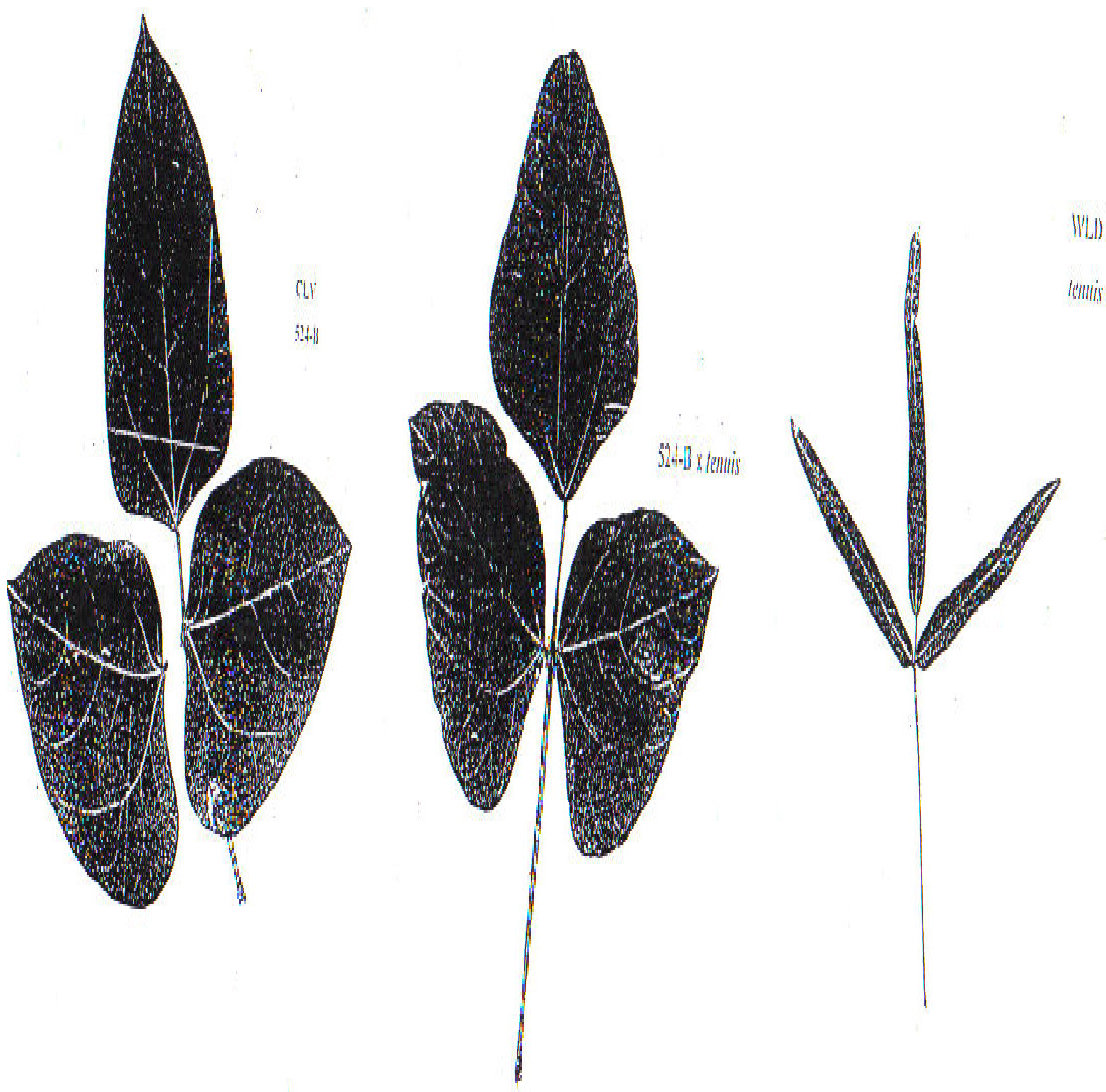

Figure 2. Case of vegetative characteristic occurred in the sterile F_1 (524-B (♀) × *tenuis* (♂)) hybrid line regarded to be near to cultivated parent.

absence because of progeny tests with them were not possible.

In studying of evolutionary processes of traits, in mutants of (524-B (♀) × *tenuis* (♂)) during the distinction between vegetative and reproductive types, these obtained individuals were morphologically near to cultivated parent, that is, the 524-B cultivar (Figure 2). The wild parent (*tenuis* subspecies) was implicated through purple flower colour, indicating probably that partial hybridisation was executed into this sterile hybrid line. Berville (2002) illustrated this kind of difference in *Helianthus* sp. studies in which hybrid plants derived from interspecific crosses between cultivated *Helianthus* (♀) × *Helianthus mollis* (♂) (that is, wild diploid and perennial species) through using molecular markers, the obtained F_1 hybrids developed fragments from wild parental strains in flower characteristics, revealing partial hybridisation occurred in these interspecific crosses. In partial.

Table 5. Germination of F_2 self-fertilised seeds produced by F_1 adult-plants of cowpea derived from reciprocal crosses between wild accessions and cultivated cowpeas (*Vigna unguiculata* (L.) Walp.), according to ISTA procedures (1976).

Hybrid combinations ♀ parents x ♂ parents	Harvested F_2 seed number/hybrid combination	Number of F_2 seeds sown/hybrid combination	Number of F_2 seeds germinated at the beginning and final periods of tests		Percent of F_2 seeds germinated (%)	Observations
			At the beginning of period of the test	At the final period of the test		
524-B x *pawekiae*	395	143	75	68	100	High germination speed
pawekiae x 524-B	756	168	98	70	100	high germination Speed
alba x 524-B	1071	193	78	115	100	High germination speed
524-B x *spontanea*	1113	188	100	88	100	High germination speed
IT84S-2049 x *baoulensis*	76	68	5	15	29.41	Low germination speed*
IT84S-2049 x *pawekiae*	1032	168	60	99	94.64	high germination speed
pawekiae x IT84S-2049	1780	188	80	108	100	High germination speed
alba x IT84S-2049	652	118	55	63	100	High germination speed
PUBESCENS x IT84S-2049	1603	193	75	118	100	High germination speed
IT84S-2049 x *spontanea*	1928	168	62	106	100	High germination speed
spontanea x IT84S-2049	1239	155	77	78	100	High germination speed
-	11645	1750	765	928	(Mean) X = 93.09%	-

*Low germination speed in this (IT84S-2049 × *Baoulensis)* hybrid line was observed at the beginning and final periods of the tests.

hybridisation, plants were nearly or completely sterile

The F_2 generation observation

We examined 11 F_2 hybrid populations derived from 11 parental different combinations. From seed number produced by natural self-fertilisation in the F_1 adult plants, the total number of F_2 plants studied in the green house was 33 individuals (Table 5).

After germination of self-fertilised seeds produced by 33 F_1 adult-plants, we detected that post-zygotic barrier mechanisms which take effect after successful fertilisation, include seed abortion and the weakness or sterility of F_1 hybrids. We have seen that F_2 plants derived from (IT84S-2049 (♀)×*baoulensis* (♂)) combination exhibited abnormalities during growth and development stages. This hybrid failed to yield viable seeds by self-pollination. Sterility identified in F_2 generation may be suggested as being due to genetic causes acting along with the effects of cryptic structural differences (Rieseberg, 2001).

Seed number obtained in F_2 generation was more important than in the F_1 generation. The difference in seed production between populations of F_2 shown that plants F_2 were productive and provided of viable seeds per pod and able to produce the next generation by natural self-pollination.

Conclusion

In producing intraspecific hybrids derived from reciprocal crosses between wild accessions and cultivated cowpea through using conventional breeding systems, artificial hybridisations yielded several crossability barriers.

Despite dysfunctions identified in embryo development during gene transfer, in intraspecific F_1 production after the germination tests, some F_1 plants obtained were fertile. Pollen fertility of these plants was normal (70%). Even with semi-fertile seeds from (524-B (♀) × *tenuis* (♂)) combinations, partial sterile plants were generated. The occurrence was induced by incomplete expression of male sterility.

The distribution of fertile plants was reduced. During characterisation, two lines derived from (IT84S-2049 (♀) × *alba* (♂) and 524-B (♀) × *alba* (♂)) restored the identical characters of the cultivars. These lines were discarded because of apomictic lines. In fertile hybrid lines, the dominance of wild genes was evident. Heterosis fixed in plants at the first generation declines in the F_2 generation because inbreeding influence. Based on intraspecific F_1 hybrids, we conclude that lines obtained in this study possessed different characters. In the second generation of segregation, it would be important to follow these works in open field under agro-ecological conditions appropriated to *V. unguiculata* species in order to elucidate inheritance of morphological characters in segregating hybrids.

With regard to the persistence of incomplete male sterility expressed in this above indicated cross, it would be advantageous to develop suitable restorer lines with the desirable recombination of morphological characteristics for exploitation of hybrid vigour.

ACKNOWLEDGEMENTS

We thank J. Geinaert for taking care of the plants in the greenhouse and for technical help. This work was entirely supported by Gent Universiteit (België).

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