Description of 90 inbred lines of castor plant (Ricinus communis L.)

Marco Baldanzi • Mirina Luiza Myczkowski • Mariangela Salvini • Mario Macchia

Received: 5 March 2014 / Accepted: 16 June 2014 / Published online: 18 July 2014 - Springer Science+Business Media Dordrecht 2014

Abstract The research describes the field comparison of 90 inbred lines of castor plant derived from both selected and wild germplasm. It was carried out in central-western Italy. An important aim of this work was to describe each inbred line based on 19 morphological traits concerning stem, leaves, racemes and capsules and then to suggest a list of descriptors to International Union for the Protection of New Varieties of Plants as to conduct the Distinctness, Uniformity and Stability test also on the castor plant. The plants in the field were grown at wide distances to avoid competition and enable observation of the growth habit, particularly the specific capacity of branching. An additional characterization of the inbred lines was obtained measuring 7 quantitative traits related to main stem and first raceme; the number of racemes per plant was used to quantify the plant branching. The results allowed distinguishing almost all the genotypes using only the morphological traits. Nectaries at the node, emergences on the stem and

M. Baldanzi \cdot M. Macchia (\boxtimes) Dipartimento di Scienze Agrarie, Alimentari e Agroambientali, Universita` di Pisa, Via del Borghetto 80, 56124 Pisa, Italy e-mail: mario.macchia@unipi.it

M. L. Myczkowski Departamento de Produção Vegetal, UNESP, Câmpus de Botucatu, São Paulo, Brazil

M. Salvini

Scuola Normale Superiore, Piazza dei Cavalieri 7, 56126 Pisa, Italy

petioles, colour of nectaries on petiole resulted important plant descriptors. The two pairs of inbred lines (Tor87#9 vs. Tor87#83 and Pod87#255Hy2 vs. Rot95#55-23) were distinguished thanks to the quantitative traits. Based on the morphological traits, two UPGMA dendrograms, one for the dwarf and one for the normal genotypes, were characterized and the resulting clusters better explained the relationships among the various inbred lines. Six genotypes (Pod87#389, Tor87#81A, Tor87#220B, Tor87#287, Tor87#287Hy, and Liba21) resulted unable to flower in the field; in these inbred lines the induction to flower is particularly influenced by the environmental growth conditions. Regarding the branching ability, the strong apical dominance of two inbred lines (Pod94#31-2 and Pod93#211) obtained from previous breeding programs was confirmed and it was possible to detect other interesting genotypes (Pod87#287A, Pod87#287B, Tor86#67). The several inbred lines described herein showed a wide range of phenotypes that might be useful in various fields of research.

Keywords DUS test · Metric traits · Plant descriptors · Plant morphology · UPGMA · UPOV

Introduction

The worldwide cultivated area of castor plant (Ricinus communis L.) remained stable at around 1.4 million hectares over the last 50 years, but total seed production doubled from 0.7 to 1.4 million Mg due to an ever increasing grain yield. The positive trend in seed production confirms the importance of this industrial oil crop and is encouraging for research programs such as recent sequencing projects (Chan et al. [2010](#page-19-0)).

The mono-specific genus Ricinus has a very high variability, in wild ecotypes as well in breeding genotypes. The plant architecture varies from shrubs to small tree individuals; the colour of the stem and leaves can vary from green to deep purple, resulting in several combinations especially with respect to the different amount of wax (bloom); the inflorescence or raceme may be more or less compact and have a different size of the capsules containing the seed, which in turn may differ in size, colour and marbling.

Considering the open pollination reproductive system of *Ricinus*, repeated generations of selfing is one simple method to fix and control the phenotypic variability (Fehr [1987;](#page-19-0) Atsmon [1989;](#page-19-0) Auld et al. [2009\)](#page-19-0). Besides, breeding programs selecting few or only one trait run the risk of genetic drift (Cowling [2013\)](#page-19-0). The same risk would be run if our breeding program aimed only at the selection of non-branching castor plants. Thus we started a program with the goal to recover as many as possible plant morphology traits from the genetic material previously obtained at the former Institute of Agronomy and Crops—University of Pisa from some varieties and hybrids originating in the USA and in East Europe. To these breeding stocks, several other accessions collected at different wild sites or from private gardens were added. The selfpollination process was repeated for ten generations, without selection, and ultimately 90 inbred lines were obtained. Some difficulties encountered during the process of self-pollination are reported here and recommendations thereupon are provided for breeders.

Another aim of this work was to obtain a description of morphological traits so as to distinguish every inbred line obtained. Moreover, flanking the work of the Government of India (2005) (2005) , this paper could be another contribute to implement a 'list of descriptors' to use in a Distinctness, Uniformity and Stability (DUS) test, still missing for the castor plant in the test guidelines of International Union for the Protection of New Varieties of Plants (UPOV).

Morphological description in the field and evaluation of some agronomic traits of the inbred lines were carried out on well spaced plants. Usually the growth habit is a good descriptor and must be observed on plants cultivated with adequate spacing, thus removing any competition. Besides, since the branching in castor plant represents an obstacle to the objective of an annual plant (Baldanzi et al. [2003\)](#page-19-0), having well spaced plants was useful for determining the branching habit of each inbred line.

Materials and methods

Self-pollination

Ten generations of selfing were carried out and only one plant per generation per genotype was grown. Three or seed, after hand-removing their hulls, were placed to germinate between two sheets of filter paper soaked with tap water, in a Petri dish, at room temperature. After germination, all the germinated seed were placed in plastic pots, with dimensions $15 \times 15 \times 25$ cm, filled with a substratum composed of a mix of potting soil and perlite (2/1, v/v) and with a controlled release fertiliser. After the emergence of the seedlings, only one was kept, thinning the others.

In the winter months, from January to March, the plants were grown in a growth chamber, with artificial light and a dark period of 8 h. In the months of April, November and December the plants were grown in a greenhouse while in the warm season, from May up to October, they were placed outdoors.

The self-pollination was carried out by enclosing the first raceme in a paper bag (Brigham [1980](#page-19-0)). Only the main stem was left growing because the axillary buds were removed. For each generation, after selfing, the first mature seed were used immediately to start the new generation while all the other seed were kept in paper bags at the temperature of 4° C as a reserve.

Some genotypes quickly reached the tenth generation of selfing, with an average of 2–3 generations per year. Others were slower, mainly due to a greater sensitivity to photoperiod resulting in a longer cycle. When the selfing process for all the genotypes was over, 90 inbred lines were obtained (Table [1\)](#page-2-0):

71 inbred lines derived from the previous germplasm of the former 'Istituto di Agronomia generale e Coltivazioni erbacee—Università di Pisa'; essentially that germplasm originated from bulk of a few foreign

For the 17 inbred lines derived from wild or garden plants, the country of origin is shown between brackets

genotypes: Baker, CNES-1, Coral, Hale, Hazera 22, Lynn, McNair 506, Novisad, Smarald;

17 inbred lines derived from samples collected in the wild and from garden plants (see Table 1 for the country of origin);

The two inbred lines 'Liba16' and 'Liba21' derived from the population 'Liba', selected at the University of Bari, Italy.

For the field comparison, the hybrid 'Negus' from Italy (former Istituto Sperimentale Colture Industriali—Osimo) was used as tester.

In some lines a plant with only female flowers in the primary raceme ('pistillate') could appear: in this case it was either fertilized with pollen from another generic line, or with pollen, sometimes refrigerated, from a specific line with high apical dominance. In this paper, the genotype names containing 'Hy' mean that they were derived from pollination of a 'pistillate' plant.

To control the Tetranychus urticae mite infestation, which can seriously infest the castor plants in the greenhouse or growth chamber (Brigham [1980\)](#page-19-0), at first an acaricide was used, but later the predator mite Phytoseiulus persimilis was preferred. In the controlled environment, even little insects like Heliothrips haemorrhoidalis and Thrips tabaci could always be insidious. In the greenhouse, two species of Lepidoptera (Noctuidae) were other minor pests: Heliothis armigera and Dysgonia algira, this latter not reported in Kolte ([1995\)](#page-19-0).

During the outdoor phase we observed ants coming and going on the extra-floral nectaries of non-waxy plants. We had to protect the young seedlings transplanted into pots because the ants were producing hard lacerations while feeding on the seedling exudates (Wackers et al. [2001](#page-20-0)). This behavior appeared less frequent in field conditions but it may be that in the natural habitats, wild castor plant may be susceptible to other species of ants.

Description

The description in the field of the inbred lines was carried on during the Summer season at the 'Rottaia' Experimental Centre, located at San Piero a Grado, Pisa, central-western Italy. The genotypes were arranged in a randomized complete block design with four replications of ten plants per plot.

The manual sowing was performed at the end of May. The emergence of seedlings was complete in the first half of June. Each plant was obtained sowing three or four seed and after the thinning only one seedling was left. The plants were spaced 2×2 m: these distances were chosen to prevent competition among individuals, allowing the free branching of each plant.

Weed control was achieved by mechanical weeding and manual hoeing. No irrigation was necessary.

The ripening racemes of dehiscent genotypes were encased in paper bags to avoid ripe seed dispersal. At harvest time, all the first racemes were picked to carry

Table 2 List of morphology traits analysed in 91 genotypes of castor plant (Ricinus communis)

Characteristics	States of expression and corresponding notes
Plant: anthocyanin colour of hypocotyl	Absent = 1, present = 9
Plant: dwarf internode	Absent $= 1$, present $= 2$
Plant: stem colour	Green = 1, green with anthocyanic shades = 2, red = 3
Plant: wax on stem	Absent = 1, present = 2
Plant: nectaries at the node	Absent = 1, few = 3, many = 5
Plant: emergences on stem	Absent = 1, few = 3, many = 5
Leaf: colour	Green $= 1$, anthocyanic $= 2$
Leaf: wax	Absent $= 1$, present $= 2$
Leaf: hairs	Absent = 1, present = 2
Leaf: colour of nectaries on petiole.	Green = 1, yellow = 2, red = 3
Flower: tepals colour	Green $= 1$, anthocyanic $= 2$
Panicle: shape	Conic = 1, near-conic = 3, near-oval = 5, oval = 7
Panicle: density	$Lax = 1$, medium = 3, compact = 5
Capsule: stalk or peduncle	Short = 1, medium = 3, $long = 5$
Capsule: colour	Green = 1, green with anthocyanic shades = 2, red = 3
Capsule: spines	Absent = 1, short = 2, medium = 3, long = 4
Capsule: epicarp	Adherent = 1, peeling of $f = 2$
Capsule: shattering	Absent = 1, weak = 2, strong = 3
Seed: size	Small = 1, medium = 3, large = 5

out the following operations: the raceme length was measured on only the section with capsules; then these latter were detached and weighed. In the larger racemes, after weighing all the capsules, only a sample of about 200 g was retained to thresh. The capsules were threshed by a special electric machine equipped with two rubber dishes.

Nineteen morphological traits were recorded (Table 2). Each state of expression is allocated a corresponding numerical note for ease of recording of data and for the production and exchange of the description (UPOV method).

An explanation is necessary for the nectaries at the node: 'few' means 2–4 nectaries on each of the two distal points of the connection between stem and petiole, whereas 'many' means that more nectaries are present.

To obtain a branching diagram showing the relationship between the analysed genotypes, two matrices of similarity were computed according to the aforesaid morphological traits, the first matrix for the 22 dwarf genotypes and the second one for the 69 normal genotypes.

The similarity coefficients were computed as.

$Sij = a/(a + b)$

where Sij is the similarity between two genotypes i and j ; a is the number of traits present in both i and j ; and b is the number of traits present either in i or j .

From the similarity coefficients, the distance was calculated for each pair of lines (distance $= 1$ similarity). Afterward, the distance matrices were used entered in the Neighbour programme of the Phylogeny Inference Package (PHYLIP), Version 3.69 (Felsenstein [1989\)](#page-19-0), using the Unweighted Pair Group Method with Arithmetic Mean of clustering (UPGMA) model.

Moreover, the following quantitative traits were counted or measured:

number of nodes on the main stem;

flowering time (no. of days from emergence);

plant height (cm) on the main stem;

number of racemes per plant;

on first raceme, length (cm) of part with capsules;

seed production on first raceme (g);

1000-seed weight (g).

The resulting data were analysed by Anova and the Least Significant Differences were calculated to compare the means.

Results

The weather during the field test was a typical Mediterranean summer, enabling the proper development of the plants up to Autumn harvest.

Six genotypes (Pod87#389, Tor87#81A, Tor87#220B, Tor87#287, Tor87#287Hy, and Liba21), probably due to their lush vegetative growth, were unable to flower, continuing to produce only leaves. For these six lines, the morphology traits of the vegetative phase were investigated in the field whereas for the reproductive traits data collected during the 10 generations of selfing were shown.

Considering the morphology traits, the 91 genotypes were sorted by grouping together those with the same response, trait by trait (Table [3](#page-5-0)). This type of sorting allowed to verify whether each genotype could be distinguished from all the others, at least by one trait. The high number of combinations for the various morphology traits made distinguishing possible for all the inbred lines, except only the following two pairs of seemingly identical genotypes: Tor87#9 and Tor87#83; Pod87#255Hy2 and Rot95#55-23.

Plant traits

The hypocotyl was more or less anthocyanin coloured in all the inbred lines tested, so this trait was not shown in Table [3](#page-5-0).

Twenty-one inbred lines and the tester resulted with a dwarf internode, grouped all together in the first 22 rows of Table [3](#page-5-0).

The anthocyanin or reddish coloured stem appeared frequently (30 genotypes), especially among those inbred lines derived from the wild types. Fourteen lines showed a green stem in young plants but reddish in adult plants, as indicated by the note '2'.

About half of the inbred lines (48/91) showed a waxy coating on the stem as well on the petiole.

Usually several or many nectaries were present around the node ('5'); thirty-four lines had only a few nectaries ('3'), and only two lines had no nectaries.

The emergences were observed in only six genotypes, with lower $('3')$ or greater $('5')$ density.

Usually the characteristics observed on the stem were also present on the petioles and on the midribs in the lower side of the leaves.

Leaf traits

The reddish leaves $(2')$ were observed only in nine inbred lines, among which six derived from wild red types, especially 'Glasgow500' which turned out purple (amaranth) in all its organs except the anthers.

Wax (bloom) was observed only on the lower side in five inbred lines and especially in young leaves.

Sparse hairs on the upper lamina, easily detectable on the young unexpanded leaves, was the rule and only nine inbred lines did not present hairs.

Nectaries on the petiole: always present at the joint with the lower lamina, generally in pairs, appeared in different forms and colours (green, yellow, red) depending on the line. Therefore in all the rows of the Table [3](#page-5-0) the corresponding notes were shown.

Flower

Before the opening of the male and female flowers, the stamens and the ovary are protected by the tepals which, usually green (1) , in the red stem genotypes could be dotted red ('1–2') or entirely coloured pink or red ('2'). Male flowers and female flowers showed tepals of the same colour, except in three genotypes (Tor87#212, 'Bordighera', 'Pantelleria').

Generally, the branched stamen filaments are colourless, but anthocyanins were found only in two wild types, 'Glasgow 500' and 'Rosignano'.

Panicle traits

The conical shape arises from a gradient in the number of capsules, which is decreasing from the bottom to the tip of the raceme. In our collection there are 49 genotypes with conic racemes ('1'); twenty-seven genotypes showed an oval raceme ('7'); the other 15 genotypes had intermediate shapes, near-conic ('3') or near-oval ('5').

The density (compactness) of the raceme was not very variable: only six inbred lines (Pod87#253, Pod87#762HyB, Tor87#270, Tor87#287, Tor87#295, and Orosei) had compact racemes ('5') and 13 lines showed lax racemes $(1')$.

Capsule traits

Twenty-four genotypes showed capsules with longer ('5') stalks (peduncle) than average ('3') and only

Seed

Capsule

Panicle

Flower

Stem

Genotype

(green = 1, green with anthocyanic shades = 2, red = 3); spines (absent = 1, short = 2, medium = 3, long = 4); epicarp (adherent = 1, peeling off = 2); shattering

 $short =$ \widehat{D} $\frac{1}{1}$ \parallel 3, large (absent \parallel

3); spines 1, medium

 \parallel $\overline{\mathbf{u}}$ $_{\rm red}$

 \mathcal{L}_{i}

anthocyanic shades $=$ \parallel

green with Ϊï

> $\overline{\mathbf{u}}$ absent (green

 $long =$ \mathfrak{S}

= 4); epicarp (adherent = 1, peeling off = 2); shattering

(absent $= 1$, weak $= 2$, strong $= 3$). Seed: size (small $= 1$, medium $= 3$, large $= 5$)

2, strong

weak $\ddot{ }$ \overline{a} \parallel

3). Seed: size (small

Genotype Stem Leaf Flower Panicle Capsule Seed

Leaf

three genotypes (Pod87#734, Tor87#49Hy1, and Tor87#49Hy2) resulted with shorter stalks ('1').

In general the capsules were green ('1'); in some genotypes with red stem, the capsules showed the anthocyanin staining at three intensities: green dotted with red $('2')$, pink or red $('3')$.

Normally, spines were present: longer in 11 genotypes $(4')$ and shorter $(2')$ in three others. Only six lines presented smooth capsules ('1').

Ten inbred lines showed a peeling off epicarp, a stable trait clearly visible during the threshing of the capsules, when other differences were also observed: in the following 21 genotypes (Cscc86#116iii, Cscc86#116iiiHy1, Cscc86#116iiiHy3, Haz87#26, Haz87#89vnc, Pod87#243BHy, Pod87#255, Pod87#255Hy1, Pod87#255Hy2, Pod87#287B, Pod87#342A, Pod87#746, Tor87#9- HyB, Tor87#220, Tor87#220A1Hy, Pod94#31-2, Rot95#55-23, Beja, Oristano, Pappiana, and Tunisia) the capsules were easier to thresh than the average, while those with a hard capsule shell (pericarp) being more difficult to thresh (Cscc86#134A, Tor86#67, Pod87#734, Pod87#734Hy, Pod93#507, Rot95#79-19). In this regard, all the wild types with small seed ('*microspermum*') were very hard to thresh.

Usually the inbred lines were non-shattering ('1') although some genotypes showed a weak opening of the capsules ('2') and occasionally the seed dropped. A mechanism of scattering ('3') was observed in those lines derived from the wild types where seed flew away; sometimes the three cocci were disjointed but each seed remained enclosed in its coccus.

Seed

Most of the inbred lines had seed of medium size, 12–15 mm long and 8–10 mm wide. Large seed were observed in the inbred line 'Casacc86#134A' and especially in the wild type 'Pantelleria' $(22 \times 17 \text{ mm})$ which belongs to the var. 'macrospermum'. The smallest seed ('1') were observed in the inbred lines derived from the wild types (var. 'microspermum') with size of about 8×5 mm.

Two distinct dendrograms following UPGMA, one for the 22 dwarf genotypes (Fig. [1](#page-9-0)) and one for the 69 'normal' genotypes (Fig. [2](#page-9-0)) were outlined.

The 22 dwarf genotypes formed an articulated dendrogram with three main clusters: three inbred lines (node 18) were separated from all the others

(node 20) which in turn formed two clusters (nodes 19 and 16). The node #1 carried the two inbred lines (Tor87#9 and Tor87#83) which did not turn out to be distinct on the basis of their morphological traits. The genotypes with red stems were grouped nearby (nodes 9, 11 and 13) except Pod87#734Hy. The several nodes did correspond with the grouping shown in the Table [3.](#page-5-0)

The dendrogram of the 69 'normal' genotypes was much more complex. A little cluster of five inbred lines (node 65) was separate from all the others, presenting the most differences among all those examined. At node 63, all the inbred lines, derived from the wild types with red stem, were clustered. The node 60 carried four lines which were derived from wild types with green stem (Capo Nero, Capo Verde, Creta and Pantelleria). On the other hand, the cluster at node 64 gathered most part of the inbred lines, and the cluster at node 62 was especially interesting as it enclosed 9 inbred lines deriving, directly or not, from

Fig. 2 UPGMA tree showing relationships among the $69\blacktriangleright$ 'normal' genotypes. The tree was drawn by the program Neighbor (PHYLIP package 3.69 version), where the UPGMA option was chosen, on the basis of a distance matrix manually generated from the descriptors data of Table [3](#page-5-0)

the selection for the non-branching trait (Baldanzi and Pugliesi [1998\)](#page-19-0).

Quantitative traits were analysed on 85 genotypes because, as stated above, six 'vegetative' inbred lines yielded no relevant data. For each trait the genotypes mean values are shown in Table [4](#page-12-0).

On the main stem we counted from 6 to over 24 nodes depending on the genotype. More than 25 genotypes had 12–13 nodes.

The number of days from emergence until flowering of the first raceme ranged from 40 to 85 days with a mean of 58 days. Many genotypes (37) bloomed at about 60 days and ten inbred lines were very early with mean values of 40 to 45 days.

Fig. 1 UPGMA tree showing relationships among the 22 dwarf genotypes. The tree was drawn by the program Neighbor (PHYLIP package 3.69 version), where the UPGMA option was chosen, on the basis of a distance matrix manually generated from the descriptors data of Table [3](#page-5-0)

Fig. 2 continued

The plant height, up to the distal end of the first raceme, ranged from 30 cm to two meters in the 'normal' genotypes and from 30 to 115 cm in the dwarf genotypes. The average plant height of the normal and dwarf genotypes were 105 and 64 cm respectively.

The number of racemes per plant, in addition to the first raceme, ranged from two to over 30. Only five genotypes produced the lowest average number of racemes (≤ 3) . The selected inbred lines of the series Pod93, Pod94 and Rot95, derived from the pedigree selection method aimed to lower the branching vigour

Table 4 List of the 85 genotypes with the means of quantitative traits

Table 4 List of the 85 genotypes with the means of quantitative traits

Table 4 continued

Table 4 continued

Table 4 continued

Table 4 continued

(Baldanzi and Pugliesi [1999](#page-19-0)), showed mean values significantly lower than the general mean.

On first raceme, the part with capsules was long from approximately 6 cm to almost ten times that much. Half of the genotypes were in the range of 20–40 cm around the general mean of 29 cm.

The 1000-seed weight had a wide range $(145-1,032)$ g) with a mean of almost 400 g. Most of the genotypes showed values between 300 and 500 g, a normal range among the cultivated types. Five inbred lines derived from wild genotypes (Capo Verde, Creta, Ponza, Rio Parana, Rosignano) had a mean around 150 g.

Also the seed production on the first raceme showed a wide variability ranging from nearly 10 g to 25 times as much with a mean of 85.7 g. Twenty-three genotypes were significantly higher than the general mean $(>114 \text{ g}).$

The results of quantitative traits did enable distinguishing those genotypes that were not distinct on the basis of morphology traits. The inbred line Tor87#83 showed significantly later flowering time and a higher yield than the line Tor87#9. The inbred lines Pod87#255Hy2 and Rot95#55-23 were significantly different for the plant height, the number of racemes, the raceme length, the 1000-seed weight and the seed production.

Discussion

We were unable to produce a single genotype with the green hypocotyl. This trait seems to be closely linked to a very clear stigma (Moshkin and Dvoryadkina [1986\)](#page-19-0) which never has really appeared in many years and thousands of plants screened. At most we observed light coloured pink-orange stigmas.

The 22 dwarf genotypes showed a wide variability in the morphology traits. The dwarf internode remained important for mechanical harvest as harvest desiccation requires plant height not to exceed 150 cm (Zimmerman [1958](#page-20-0); Weiss [1983](#page-20-0)). This trait does not seem unique to improved genotypes because very short internodes were observed in the 'microspermum' wild types 'Orosei', 'Ponza' and 'Rio Parana'. Also 'Pantelleria', a 'macrospermum' type, has a shortened internode.

Genetics for the stem colour was studied by several authors to shed light on the variability in the intensity of red (Moshkin and Dvoryadkina [1986\)](#page-19-0). It is a trait easily used to distinguish between genotypes: together with the next trait, it allows a useful grouping for the castor plant genotypes. The 'dark red' colour observed only in the genotype 'Glasgow500' could be added in addition to the three levels here proposed.

The waxy coating may cover the different organs of the plant to differing degrees. Our results confirmed the distinction of Kulkarni and Ramanamurthy [\(1977](#page-19-0)): (i) no-wax; (ii) single-waxy: only on stems; (iii) double-waxy: on stem, capsules, and on the lower side of leaf; (iv) triple-waxy: on stem, capsules, both upper and lower sides of leaves. The last category was not observed in our collection.

The presence of nectaries at the node can be easily identified; being a typical quantitative trait, its determination on the median node of the main stem is recommended

Usually the stems in castor plant are smooth. Here, the term 'emergences' (Esau [1965](#page-19-0)) was preferred to 'small echinate protuberances' of Kulkarni and Ramanamurthy ([1977\)](#page-19-0). It is a quantitative trait and its occurrence can be easily detected on the petiole.

Passing to the leaf traits, Moshkin and Dvoryadkina [\(1986](#page-19-0)) cited some papers dealing with the genetics of the colour for stem and leaves. It is important to note the leaf colour on well expanded leaves, because in certain red stem genotypes, only the young leaves show anthocyanin and then become green [\(http://](http://agricoop.nic.in/seedtestguide/castor.htm) [agricoop.nic.in/seedtestguide/castor.htm\)](http://agricoop.nic.in/seedtestguide/castor.htm). Red expanded leaves can be found only on genotypes with red stem, as we described here in six inbred lines derived from wild types and in three inbred lines from selected genotypes. These last indeed were derived from crosses where 'Pappiana', one of those six red wild types, was used as the male parent. Red leaves seem associated with resistance to some pests (Anjani et al. [2007](#page-19-0)).

Three other leaf traits were proposed for a list of descriptors: shape, number of lobes and laciniate margin [\(http://agricoop.nic.in/seedtestguide/castor.](http://agricoop.nic.in/seedtestguide/castor.htm) [htm](http://agricoop.nic.in/seedtestguide/castor.htm)). The leaf shape, flat or cup, is associated with the internode length: e.g. the dwarf types have cup leaves. Number of lobes and laciniate margin need an additional evaluation before to be accepted as official descriptors.

The type of nectaries at the border between the petiole and the lower side of the leaf was recorded as an unexpected trait for the distinctiveness, quite accurate because the same form always resulted on all plants of the same genotype. Moshkin and Perestova [\(1986](#page-19-0)) quoted a paper on the presence of 'functional nectar glands' on the different organs, whereas Kulkarni and Ramanamurthy [\(1977](#page-19-0)) reported a work on their genetic control: the 'red gland' is dominant over yellow. While the shape of the nectaries at the nodes is sub-globular and the colour is red or yellow, the form of the nectaries between stem and leaf is almost cuplike. This can vary in size and shape depending on the genotype. The colour ascribed refers to the bottom of this cup and the observed colours (green, yellow, red, or combinations of these) do not appear to be associated with the colour of the stem. As combinations, the edge of the 'cup' might be a different colour from the bottom. Two nectaries was counted in most genotypes, though alternatively there was only one. In rare cases there were also four nectaries (Pod87#389, Tor87#220B, Tor87#287Hy): these three genotypes belong to the six without flowering and then the more luxuriant vegetation may have increased the number of nectaries. Therefore, these nectaries are proposed as a key characteristic for the list of descriptors in castor plant. A more detailed description of their form would be helpful, with their outline and colour. As for the number of nectaries at the nodes, we suggest describing the nectaries between petiole and leaf on the leaves of the median zone of the stem when the first raceme starts flowering.

For the flowers, in addition to the colour of tepals, other three descriptors could be added: (i) male and female flowers differ for the tepals colour; (ii) the male flowers before opening up are almost spherical, as observed in the microspermum wild types; (iii) the stamen filaments are anthocyanic, as here observed in only two genotypes.

The variation of the ratio between the number of male flowers and female flowers and the presence of 'interspersed' male flowers has stimulated works that have contributed to the genetics of sex in plants (Shifriss [1960](#page-19-0); Zimmerman and Smith [1966;](#page-20-0) William and Shifriss [1967](#page-20-0)). About the ratio between male and female flowers as a descriptor, the classification of Kulkarni and Ramanamurthy [\(1977](#page-19-0)) is recommended: (i) mostly female spike, (ii) partially female spike, and (iii) mostly male spike. A fourth type has male flowers interspersed in a inflorescence of only female flowers. Almost all of the genotypes here described are of the

type (ii) while only a few lines belonged to each of the other three types. It is important to refer this ratio only to the first inflorescence.

During the selfing process, occasionally plants with only female flowers on the primary raceme appeared, which were hand fertilized with pollen of another preferably one with strong apical dominance. These plants with only female flowers are called 'pistillate' and even now stable pistillate genotypes are used for large scale commercial hybrid seed production.

The variability of the shape and density of the raceme is well known to researchers involved in castor plant. In order to simplify the use of descriptors and avoiding dubious interpretations without losing in accuracy, perhaps it is advisable to use only three forms of raceme: conic, intermediate and oval. To classify the primary raceme of a certain genotype will be more precise if the length of the raceme is considered.

The density of the raceme was easier to characterise by the three classes here used to describe our collection, respect to the classification proposed by Kulkarni and Ramanamurthy ([1977\)](#page-19-0): (i) firmly compact, (ii) compact, (iii) loose, (iv) very loose. This trait is connected to the stalk (peduncle) length of the capsule which Kulkarni and Ramanamurthy ([1977\)](#page-19-0) reported as sessile vs. elongated. The sessile capsules form a compact raceme while longer or shorter stalk yields racemes differing in compactness (Moshkin and Dvoryadkina [1986\)](#page-19-0). Branched vs. non-branched stalk of the capsule was mentioned by Kulkarni and Ramanamurthy ([1977\)](#page-19-0). Although in this trial nonbranched stalk was observed in two genotypes (Cscc86#116iiiHy1 and Beja), this trait is very questionable. In the castor plant the inflorescence is a panicle that is a composed or branched raceme. The branching of the stalk is greater in long racemes with many capsules in the lower part of the raceme, hence the conical shape. Otherwise, branching may be lesser but not completely absent. Even when the capsules are sessile, branching of the stalk (peduncle) was observed, from which the most compact racemes derive. Furthermore, in the racemes with long peduncle capsules, a medium compactness can be observed, not loose, thanks to the normal branching of the stalk.

For the capsules Popova and Moshkin ([1986\)](#page-19-0) reported six colours: green, yellow, brown, red, crimson, and violet. Although sometimes plants with pale yellow capsules were observed, it was not possible to fix this trait in any genotype. The type 'brown' was never observed and the type 'violet' should correspond to the dark red colour with wax. Kulkarni and Ramanamurthy ([1977\)](#page-19-0) reported these colours: green, prevalent among the cultivated varieties, mahogany, pink and sulfur-white. In essence, for a list of descriptors, it would be good to agree on the degrees of anthocyanin colouring. Note that for determining the colour of stems, leaves and capsules in the waxy genotypes it is more accurate to look beneath the waxy bloom.

For the spines on the capsules, variability both of the number of spines as well as their length was documented (Kulkarni and Ramanamurthy [1977](#page-19-0); Moshkin and Dvoryadkina [1986](#page-19-0)). Surely, it is advisable to note a sparse density from a normal density of spines. Moreover, assuming 5 mm as a medium length, genotypes can occur with longer or shorter spines.

The peeling off epicarp can be easily observed in spiny as well non-spiny capsules when they are dried and so it can be considered a useful descriptor. Only in the non-spiny capsules, when they are still ripening and not yet dried, the epicarp can be smooth or warty (Kulkarni and Ramanamurthy [1977](#page-19-0); Moshkin and Perestova [1986\)](#page-19-0). In the collection here presented, only six inbred lines have non-spiny capsules and all showed a smooth epicarp.

The dehiscence of the capsules is described in the literature and reported here for some inbred lines, however this trait cannot appear in the improved varieties and hybrids.

Unlike the other characteristics, Kulkarni and Ramanamurthy [\(1977](#page-19-0)) do not dwell much on the seed size. Moreover, they report how largest size as 21×13.5 mm while the seed of our '*macrospermum*' genotype 'Pantelleria' were bigger. Moshkin and Perestova [\(1986](#page-19-0)) listed two questionable values for length (30 mm!) and width (15 mm) of the seed. In the botanical classification reported by Popova and Moshkin ([1986\)](#page-19-0) we found the terms microcarpus or microspermus for 'small seed' varieties and macrocarpus or megalospermus for 'large seed' varieties. Here the terms macrospermum and microspermum were adopted referring to the wild types, usually dehiscent, for dimensions that fall outside the typical range of cultivated varieties.

The following additional seed traits may be useful as descriptors to distinguish the genotypes.

The seed shape (elongated, oval or square) as used in India [\(http://agricoop.nic.in/seedtestguide/castor.](http://agricoop.nic.in/seedtestguide/castor.htm) [htm](http://agricoop.nic.in/seedtestguide/castor.htm)) is simple to determine and stable in every genotype.

The caruncle (elaiosome) can vary in size and shape, as well as by the presence of anthocyanins. Ants attacking this organ were observed: even if they cannot carry the seed (Martins et al. [2009\)](#page-19-0), they gnaw the caruncle of seed dispersed on the ground and sometimes even the mature seed still inside the capsule.

The inner seed hull has a white film closely adhering to the endosperm (tegmen): in some genotypes this can have dark dots.

Finally, the colour and the mottling of the seed tegument could be safe descriptors because they are easily recognizable and have almost no environmental influence; the phenotypes present in [http://agricoop.](http://agricoop.nic.in/seedtestguide/castor.htm) [nic.in/seedtestguide/castor.htm](http://agricoop.nic.in/seedtestguide/castor.htm) could be a first step to define unequivocally the variegated pattern of the castor plant seed.

The two UPGMA dendrograms based on the same descriptors of the Table [3](#page-5-0) produced a more systematic diagram of our inbred lines, better highlighting their relationships and thus representing a practical useful visualization of our collection. The two Figures added more information respect to the Table [3](#page-5-0). If this one could be considered an analysis of the several observed phenotypes then the two dendrograms gave a synthesis view of the germplasm here described. That does reiterate the importance of the plant morphology traits to be used as descriptors and their fundamental nature during the DUS test.

For a better characterization of the 90 inbred lines the following seven quantitative traits were considered.

The number of nodes on the main stem is easy to determine since the nodes are easily recognizable thanks to the scars left after the fall of stipules and leaves. After days to flowering, it is a convenient estimate of the genotype's earliness. During the selection carried out by us to reduce branching (Baldanzi and Pugliesi [1998](#page-19-0)) we saw the usefulness of discarding genotypes with fewer than 11 nodes because they are more inclined to branch out. In our opinion a range of 15–20 nodes is ideal for a nonbranching genotype of castor plant.

Concerning the days required for initiation of flowering, Kulkarni and Ramanamurthy ([1977\)](#page-19-0) reported a range between 30 and 180 days depending on the genotypes and locations while in Moshkin [\(1986](#page-19-0)) the types shown are not as so late. As a descriptor it could be added to note whether the male and female flowers open at the same time, as usually happens, or not.

The plant height up to the distal tip of the first raceme was measured, and not the final plant height. Since the growth of castor plant is indeterminate, it is influenced too much by environmental conditions. On the other hand, considering the model for a nonbranching castor plant, a minimal attention is appropriate with respect to what happens beyond the first raceme. A non-branching castor plant should be similar to the cultivated sunflower, where the mass of seed production is at the top of the plant, thus the higher the more susceptible it is to lodging: we consider a height of 150 cm optimal. Concerning the 'dwarf' internode, in our opinion these genotypes are important for industrial castor plant, in the sense that they make smaller the branched plants with two–three racemes after the first, a size that also allows for chemical treatment (desiccant) of crop vegetation for the subsequent mechanical harvest. Three dwarf genotypes in our collection were over 100 cm in height because they had a long first raceme, over 40 cm.

The number of racemes per plant can be an estimate of branching intensity, a trait that obtains only in distantly spaced plants as in this trial. A feature seldom investigated by other researchers, it is very important to us and our results allowed us to identify five genotypes with very low values (≤ 3) , to be considered in subsequent breeding programs. At the end of the autumn (December), when the plants lost their foliage, the plant architecture was easily described; thus the type of branching observed and classified was as basal, median or upper (data not shown). Since we were interested in a more quantitative assessment of the branching than a qualitative one, we opted to omit this trait. Nevertheless, it could be a useful descriptor where usually branching varieties and hybrids are cultivated. Such a description of branching is, for example, used in the DUS test of sunflowers (UPOV [2000](#page-19-0)). In India, convergent or divergent branching pattern and top or basal location of branches are used as descriptors for castor plant genotypes [\(http://](http://agricoop.nic.in/seedtestguide/castor.htm) [agricoop.nic.in/seedtestguide/castor.htm\)](http://agricoop.nic.in/seedtestguide/castor.htm).

In the raceme, the length of part with capsules is highly correlated with grain production, both in the compact and in the loose racemes. As a descriptor, this measurement would be sufficient to characterize a genotype. The values shown here were indicative of a wide range of phenotypes, a feature of this species. Linking this trait with the number of racemes per plant, no genotypes appear with many long racemes, i.e., the greater lengths were found only in genotypes producing few racemes. This result should be considered important in plant breeding because it can link the selection of the non-branching types with long racemes, which have higher seed production.

The 1000-seed weight complemented the seed size trait. Some wild types, i.e., Bordighera, Capo Nero, Glasgow500, were not different from the inbred lines which had small seed i.e., Cscc86#172B, Pod87#255, Tor87#47 and Tor87#270. It should be remembered that seed too large, i.e., with a 1000-seed weight over 500 g, are more susceptible to breakage during threshing at harvest. For a list of descriptors, in order to avoid any misunderstanding, it might be more practical to classify the seed size in the following five categories according to the 1000-seed weight: very low = 1 (up to 150 g); low = 3 (150–350 g); medium $= 5$ (350–450 g); high $= 7$ (450–650 g); very high $= 9$ (more than 650 g).

Usually seed production is not used as a descriptor trait but the range of values here observed for the first raceme was really very large and could characterise the genotypes of our collection even more. It is true that seed production by only the first raceme does not indicate the potential grain yield of a genotype. However, this trait is of interest to us because our main goal is a non-branching plant precisely where seed production comes from only the first raceme. Indeed, the four most significant seed production values were found in genotypes with a number of racemes per plant of less than 10, two of which were the lines Pod94#31-2 and Pod93#211 derived from genotypes with strong apical dominance (Baldanzi and Pugliesi [1998](#page-19-0)).

As stated above it was not possible to measure the quantitative traits in the six 'vegetative' inbred lines which only if grown in pots are able to flower and produce seed; it could be interesting to examine these genotypes more in depth, especially for the relation between nitrogen and flower genes.

In conclusion, the selfing process without selection permitted us to obtain 90 inbred lines which showed a wide spectrum of several morphological traits, thus avoiding the risk of losing potentially useful genes. Also, the agronomic traits displayed a high variability and certain genotypes stood out for their interesting performance.

The usefulness of the DUS test is such that it must be applied to a species which many researchers, to varying degrees, recognise as important for both technological and scientific purposes. The number of descriptors may vary depending on the species: i.e., in sunflowers, a seed oil crop that we use as a point of reference for a model castor plant, there are 42 descriptors. Here we have listed a minimum number of traits, 19 morphological and 7 quantitative including seed production, which sufficed to distinguish almost all the 91 tested genotypes. When the castor plant becomes a better domesticated crop, probably a minor variability among the cultivated genotypes will be present and thus a greater number of descriptors will be necessary.

Compared to previous monographs on castor plant, some changes and additions to the plant morphological description were suggested. The traits and their variability reported here are not new for those who have been working for years with castor plants. Nonetheless we urge castor plant breeders to apply the UPOV so that this crop may also have an official list of descriptors.

Finally, a description and a comparison of inbred lines this different could be useful to those researchers who need homozygous material for the study of this unique species.

Acknowledgments Andrea Bertacchi, Rosanna Bertozzi, Sauro Cappagli, Maria Carrau, Salvatore Casano, Franco Cecconi, Marcello Chiavoni, Nicola Colonna, Adriana Cozzani, Davide Dattola, Mauro Durante, Grazia Fiaschi, Carlo Gatti, Alessio Macchia and Guido Pardini provided seed of the wild and garden genotypes. Roberto Canovai, Barbara Conti, Giorgio Loi, Alfio Raspi, Elisabetta Rossi and Luciano Santini determined each pest species and suggested the appropriate control methods. Antonino Onnis recommended the use of the growth chamber. Mirina Luiza Myczkowski has participated in this research thanks to a grant from the CAPES, Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior, Faculdade de Ciências Agronomicas, Unesp/Botucatu.

References

Anjani K, Pallavi M, Babu SNS (2007) Uniparental inheritance of purple leaf and the associated resistance to leafminer in castor bean. Plant Breed 126:515–520

- Atsmon D (1989) Castor. In: Röbbelen G, Downey RK, Ashri A (eds) Oil crops of the world. McGraw Hill, New York, pp 348–447
- Auld DL, Zanotto MD, McKeon T, Morris JB (2009) Castor. In: Vollmann J, Rajcan I (eds) Oil crops. Handbook of plant breeding. Springer, New York, pp 316–332
- Baldanzi M, Pugliesi C (1998) Selection for non-branching in castor, Ricinus communis L. Plant Breed 117:392–394
- Baldanzi M, Pugliesi C (1999) Searching for a non-branching plant in castor. Ital J Agron 2:127–132
- Baldanzi M, Fambrini M, Pugliesi C (2003) Redesign of the castorbean plant body plan for optimal combine harvesting. Ann App Biol 142:299–306
- Brigham RD (1980) Castor. In: Fehr WR, Hadley HH (eds) Hybridization of crop plants. ASA and CSSA Publishers, Madison, pp 235–247
- Chan AP, Crabtree J, Zhao Q, Lorenzi H, Orvis J, Puiu D, Melake-Berhan A, Jones KM, Redman J, Chen G, Cahoon EB, Gedil M, Stanke M, Haas BJ, Wortman JR, Fraser-Liggett CM, Ravel J, Rabinowicz PD (2010) Draft genome sequence of the oilseed species Ricinus communis. Nat Biotechnol 28:951–956
- Cowling WA (2013) Sustainable plant breeding. Plant Breed 132:1–9
- Department of Agriculture and Cooperation—Ministry of Agriculture—Government of India (2005) National guidelines for the conduct of tests for distinctness, uniformity and stability—Castor (Ricinus communis L.). [http://](http://agricoop.nic.in/seedtestguide/castor.htm) agricoop.nic.in/seedtestguide/castor.htm. Accessed 23 April 2014
- Esau K (1965) Plant anatomy, 2nd edn. John Wiley & Sons, Inc., New York
- Fehr WR (1987) Principles of cultivar development. Theory and technique, vol 1. Macmillan, New York
- Felsenstein J (1989) PHYLIP—Phylogeny Inference Package (Version 3.2). Cladistics 5:164–166
- Kolte SJ (1995) Castor. Diseases and crop improvement. Shipra Publications, Delhi
- Kulkarni LG, Ramanamurthy GV (1977) Castor. ICAR, New Delhi
- Martins VF, Guimaraes PR Jr, Haddad CRB, Semir J (2009) The effect of ants on the seed dispersal cycle of the typical myrmechorous Ricinus communis. Plant Ecol 205:213–222
- Moshkin VA (1986) Direction of breeding and criteria of selection. In: Moshkin VA (ed) Castor. Oxonian Press, New Delhi, pp 117–125
- Moshkin VA, Dvoryadkina AG (1986) Cytology and genetics of qualitative characteristics. In: Moshkin VA (ed) Castor. Oxonian Press, New Delhi, pp 93–103
- Moshkin VA, Perestova TA (1986) Morphology and anatomy. In: Moshkin VA (ed) Castor. Oxonian Press, New Delhi, pp 28–34
- Popova GM, Moshkin VA (1986) Botanical classification. In: Moshkin VA (ed) Castor. Oxonian Press, New Delhi, pp 11–27
- Shifriss O (1960) Conventional and unconventional systems of controlling sex variation in Ricinus. J Genet 57:361–388
- UPOV (2000) TG/81/6 Guidelines for the conduct of tests for distinctness, uniformity and stability. Sunflower (Helianthus annuus L.). Geneva, Switzerland. [http://www.upov.](http://www.upov.int/edocs/tgdocs/en/tg081.pdf) [int/edocs/tgdocs/en/tg081.pdf](http://www.upov.int/edocs/tgdocs/en/tg081.pdf)

Wackers FL, Zuber D, Wunderlin R, Keller F (2001) The effect of herbivory on temporal and spatial dynamics of foliar nectar production in cotton and castor. Ann Bot 87:365–370

Weiss EA (1983) Oilseed crops. Longman, New York

William GL, Shifriss O (1967) Interspersed sexuality in Ricinus. Genetics 57:347–356

- Zimmerman LH (1958) Castorbeans: a new oil crop for mechanized production. Adv Agron 10:257–288
- Zimmerman LH, Smith JD (1966) Production of F_1 seed in castor-beans by use of sex genes sensitive to environment. Crop Sci 6:406–409