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A review of ploidy in the genus *Prosopis* (Leguminosae)

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The genus *Prosopis* contains 44 species of trees and shrubs, the majority of which originate in the Americas. Most species are reported to be diploid with a somatic chromosome number of $2n = 28$, with rare reports of polyploidy, although it was thought that these may represent polysomaty in root tissues. However, flow cytometry has recently indicated that *P. juliflora* is entirely tetraploid with a somatic number of $2n = 56$.

In order to clarify the situation, a full review of ploidy in *Prosopis* was undertaken, the first of its kind. The ploidy levels of 124 samples of *Prosopis* from 21 countries including both the natural and introduced ranges were analysed using flow cytometry. Additionally, a comprehensive literature review was carried out, examining 305 published ploidy values and covering 32 of the 44 species of *Prosopis*.

Flow cytometry analysis suggested that *P. juliflora* is the only tetraploid species, with a somatic chromosome number of $2n = 4x = 56$, whilst the remainder of the species analysed were diploid with a somatic chromosome number of $2n = 2x = 28$, including the first report for *P. articulata* ($2n = 28$).

A critical review of published ploidy values shows that all species of *Prosopis* were reported to be entirely diploid, except *P. glandulosa*, *P. juliflora* and *P. koelziana*, for which both diploid and tetraploid values have been recorded.

ADDITIONAL KEYWORDS: chromosome number – diploid - flow cytometry – new estimate – polyploidy - tetraploid – triploid

INTRODUCTION

The genus *Prosopis* contains 44 species of trees and shrubs. All are nitrogen-fixing and most are fast-growing, hardy and drought tolerant. Most species originate in the Americas, with only four outside this region, occurring in Africa and Asia (Burkart, 1976). Economically-important species of *Prosopis* have been introduced worldwide, including the only truly tropical New World species *P. juliflora* (Sw.) DC and *P. pallida* (Humb. & Bonpl. ex. Willd.) Kunth, as well as sub-tropical species such as *P. glandulosa* Torrey and *P. chilensis* Mol. Stuntz emend. Burkart, from North and South America respectively.

Prosopis trees and shrubs can provide a wide range of products from various parts of the plant. Timber can be used in the construction industry and wood for tools, fencing and fuel. Pods can provide a range of food items, such as syrups, flour and also alcoholic drinks (Pasiecznik *et al*., 2001). Trees and shrubs can also be used in agriculture to provide fodder and nectar as well as to provide shade and shelterbelts. The introduction of these economically important species of *Prosopis* in the arid parts of the world has provided valuable resources for some of the poorest communities. However these introductions have not met with universal approval. In some parts of the world a few species have become problem weeds and steps have been taken to manage these populations and eradicate invasive species (Pasiecznik *et al*., 2001).

Closely-related species of *Prosopis* have similar morphology and identification can be difficult, especially in the absence of flowers and fruit. Additionally, the environment can have considerable effects upon plant form, leaf characteristics and growth (Pasiecznik *et al*., 2001) and closely-related species are known to hybridise readily (Hunziker *et al*., 1986), adding to the problems of identification. In addition, the taxonomy of the genus is complicated and the classification has been re-organised a number of times since the genus was first described. The most recent re-classification was that of Burkart (1976), who raised and lowered the ranks of a number of taxa compared with previous taxonomic treatments and problems have occurred when full species and variety names have not been quoted. For instance, prior to Burkart (1976) the species now known as *P. glandulosa* Torrey was regarded as a subspecies of *P. juliflora (P. juliflora* (Sw.) DC var. *glandulosa* (Torrey) Cockerell). Thus, introduced populations of *P. glandulosa* may be identified currently as *P. glandulosa* Torrey, *P. juliflora* (Sw.) DC var. *glandulosa* (Torrey) Cockerell, *P. juliflora* or indeed simply as *Prosopis* sp. The identity of many naturalised stands of *Prosopis* is not known.

Our recent research has focussed on the identification, management and resource potential of the genus in developing countries (Cadoret, Pasiecznik & Harris, 2000; Pasiecznik *et al*., 2001; Harris *et al.*, 2003). A method to differentiate the closely related species, *P. juliflora* and *P. pallida* using leaf morphology and ploidy has been described (Harris *et al*., 2003) and has been used to develop keys for identification of these species from their foliar characters alone (Pasiecznik, Harris & Smith, 2004).

Many of the data available to date suggest that *Prosopis* is essentially a diploid genus with a somatic chromosome number of $2n = 28$ (Burkart, 1976; Pasiecznik *et al*., 2001), but there have been some reports of polyploidy (Cherubini, 1954; Hunziker *et al*., 1975; Burkart, 1976). Harris *et al*. (2003) suggested that, whilst *P. pallida* is a diploid species, *P. juliflora* is tetraploid, providing a reliable means of distinguishing between these two closely-related species. This raises the question of whether *P. juliflora* is the only normally tetraploid species in the genus. In order to answer this, additional samples of *Prosopis* from world-wide collections were identified using foliar characters as described by Harris *et al.* (2003) and their ploidy levels were determined. These primary data were supported by a detailed literature search of previous estimations in order to provide a comprehensive review of ploidy in the genus *Prosopis*.

MATERIAL AND METHODS

Samples of a number of species were obtained from 21 countries, both from their natural and introduced ranges (Tables 1-3). Collection sites and origin, if known of mature leaves or seeds are shown in Tables 1-3.

After being mechanically scarified, seeds were placed on moist filter paper in petri dishes and allowed to germinate in the dark at 25 ºC for 3-4 days. Once germinated, they were transferred to pots of compost and kept in a greenhouse at a temperature of 18-22ºC watered every 2-3 days and fed with Phostrogen weekly.

PLOIDY ANALYSIS

The DNA content of cells was determined by flow cytometry and related to chromosome counts from root tip squashes to indicate ploidy levels. Root tip squashes were prepared from a number of samples whose DNA content differed and which were presumed from the flow cytometry results to be diploid or tetraploid.

Flow cytometry was carried out using fresh leaves collected either from mature trees or from seedlings raised in the greenhouse. Chromosome counts were carried out using root tips, also obtained from seedlings raised in the greenhouse.

FLOW CYTOMETRY

For flow cytometry, three replicates of fresh leaf material were prepared from each sample, each replicate providing enough for analysis. Only a few cm^3 of leaf material was required for this method. Thus, each replicate contained between one and five leaves depending upon size.

Replicates were sealed in a labelled plastic bag with moist filter paper to keep the samples fresh, and sent for ploidy analysis to Plant Cytometry Services, Schijndel, The Netherlands. The laboratory followed a modified method of Arumuganathan & Earle (1991). Leaf samples are placed ice cold buffer solution in a petri dish and co-chopped with an internal standard. In this analysis, iceberg lettuce (*Lactuca sativa* var. *capitata*) was used as an internal standard. The suspension was passed through a nylon filter to remove any large remnants of leaf tissue and to isolate the nuclei. After staining with DAPI (4', 6-diamidino-2-phenylindole), a dye which fluoresces at 465nm, the suspension was then passed through a cytometer (Partec PAS II). The flow cytometer measures the fluorescence of the stained nuclei and the output of the instrument is then processed by computer to produce DNA histograms. The ploidy of the samples was then estimated from the histograms, by comparing the peak value of the internal standard with that of the sample. The relationship between chromosome counts and ratio of sample to standard peaks obtained by flow cytometry has been previously well established in *Prosopis* (Harris *et al*., 2003).

ROOT TIP SQUASH METHOD

Root tips, approximately 15mm in length, were collected from 3-5-day-old seedlings and immersed in 0.002M 8-hydroxyquinoline for 3.5 h at room temperature.

They were fixed in 1:3 acetic acid:ethanol and left at room temperature for 1 hour. Root tips were then hydrolysed in 1M HCl for 20 min at 60 °C before staining with basic fuschin for 3.5-4 h, after staining; root tips were then squashed on slides.

RESULTS AND DISCUSSION

PLOIDY ANALYSIS

In total, 124 samples were analysed using flow cytometry and were found to be either diploid ($2n = 2x = 28$) or tetraploid ($2n = 4x = 56$) (Tables 1-3).

Nine of the ten *Prosopis* species examined were diploid. Five samples of the North American species; *P. articulata*, *P. glandulosa*, *P. laevigata* and *P. velutina*, obtained from their natural ranges in Mexico and the USA, were diploid, as were all samples from introduced populations of these species. Similarly, each of the samples of *P. affinis*, *P. caldenia*, *P. chilensis* and *P. pallida*, species that occur naturally in South America, had a somatic chromosome number of $2n = 28$. Samples of *P. cineraria* collected in India were the only representatives of the Old World species of *Prosopis* examined and all were diploid.

P. juliflora occurs naturally in both North and South America. In this investigation the 65 samples of *P. juliflora* analysed were predominantly tetraploid and no diploid samples were obtained. While some triploids were discovered, these were exceptional cases discussed in more detail below. It was concluded therefore, that *P. juliflora* is an entirely tetraploid species, findings which agree with those of Harris *et al.* (2003).

A few triploids ($2n = 3x = 42$) were identified (Table 2). Triploid seedlings were obtained from two samples for which mature leaves were also available; these mature trees were identified as *P. juliflora* from foliar characteristics (L037, Jordan and L047, India). Triploid seedlings were also identified from three seed accessions (Table 3), two from the Cape Verde Islands (L066 & L075) and one from a commercial seed supplier (L083), for which the origin is unknown. Triploids were first reported by Harris *et al*. (2003). Hybridisation between *Prosopis* species is thought to be frequent and has been reported previously (Hunziker *et al.*, 1975). For the two triploids obtained from Jordan (L037) and India (L047) where one parent is known, it is thought that they are hybrids of tetraploid *P. juliflora* and a diploid species, possibly *P. pallida* or *P. chilensis*, which are known to occur in

India and Jordan respectively. Mature leaves of triploid hybrids between *P. juliflora* and *P. pallida* have not yet been studied, and it is not clear whether their foliar characters would be intermediate, as observed in hybrids between other *Prosopis* species (Hunziker *et al.*, 1975; Naranjo, Poggio & Zieger, 1984). In this study, none of the seed collected in the native ranges produced triploid seedlings.

LITERATURE REVIEW

A comprehensive literature search was carried out to compare values obtained in this investigation with those published previously. A summary of values obtained is shown in Table 4. In all, 305 values for 32 species of *Prosopis* were obtained, with the earliest being that recorded by Covas & Schnack (1946). Of these 305 records, approximately half were in secondary references; that is lists or databases of chromosome numbers for the genus, so were not new data, but rather compilations of existing data. Ploidy values listed by these secondary sources are omitted in the main part of the table, but are referenced in column 6 of the table. Thus, only values obtained by the primary source are included in Table 4.

Some ploidy values obtained have been omitted. Ploidy values for *P. insularum* and also *P. insularum* subsp. *novoguineensis* (Breteler, 1960, reported in Hunziker *et al*., 1975) and *P. striata* (Castronovo, 1946, reported in Darlington & Wylie, 1955) have been omitted since these are no longer considered to be part of the genus and are now classified as *Piptadenia novo-guineensis* Warb. and *Prosopidastrum globosum* (Gillies ex Hook. & Arn.) Burkart respectively (Burkart, 1976). Ploidy values for *P. lampa* Willd. have also been omitted. This species is not included in the classification of Burkart (1976), nor does it appear on the International Plant Name Index (IPNI, September 2004). It appears that this taxon was first cited by Bukhari (1997b, 1997c) and is probably a typographical error, as Lampa is a location in Chile. However, a sample of '*P. lampa*' was obtained from DANIDAFSC and found to be diploid in this study.

Thus, in all, 152 primary references were obtained for 32 of the 44 species described by Burkart (1976). No values were found in the literature for the following twelve species of *Prosopis*: *P. abbreviata* Benth., *P. articulata* S. Watson, *P. burkatii* Muñoz, *P. elata* (Burkart) Burkart, *P. calingastana* Burkart, *P. castellanosii* Burkart, *P. fiebrigii* Harms, *P. palmeri* S. Watson, *P. pugionata* Burkart, *P. rojasiana* Burkart, *P. rubriflora* Hassl. and *P. tamaulipana* Burkart. Several of these species are isolated and/or rare, so the lack of data is not surprising. In the present study, one sample of *P. articulata* S. Watson was analysed and found to be diploid ($2n = 28$), the first report for this species (Table 3).

The base number for the genus is generally considered to be $x = 14$, and indeed most somatic values are reported as $2n = 28$, 56, or very occasionally 112. However a few values with a base number of $x = 13$ have been recorded, all from the Indian sub-continent. Chromosome numbers of $n = 26$ for *P. cineraria* were recorded by Kumari, Saggoo & Kaur (1989) and $2n = 26$ for *P. glandulosa* Torr. by Ramanathan (1950) and for *P. juliflora* values of $2n = 52$ were reported by Sampath & Ramanathan (1949), Kumari & Bir (1985) and Ohri, 1996, recorded in Bennett & Leitch, 2004). In addition, Bir & Sidhu (1967) found a haploid number of $n = 13$ for *P. juliflora*. Ploidy values with a base number of $x = 14$ have been recorded from this region for various species of *Prosopis* by Hunziker *et al.*, (1975), Gill *et al*., (1984), Bandyopadyay *et al*., (1990), Singhal, Bir & Sidhu (1990) and Bukhari, (1997a, 1997c). It is not clear whether the uncharacteristic x=13 values are due to speciation within populations found in this region, or are due to mis-identification or experimental error.

Of those 32 species that have published ploidy values, the large majority were diploid with a $2n$ value of 28, while some with ploidy values other than this were found, most of these being tetraploid ($2n = 56$). Twenty-seven species were wholly diploid, the remaining five species had variable ploidy and none was wholly tetraploid. As well as having a number of diploid values recorded, five species also have tetraploid values recorded by the following authors; *P. cineraria* (Kumari *et al*., 1989), *P. chilensis* (Bukhari, 1997b, 1997c), *P. glandulosa* (Gill *et al*., 1984; Singhal *et al*., 1990), *P. juliflora* (Sampath & Ramanathan, 1949; Atchinson, 1951; Hunziker *et al*., 1975; Kumari & Bir, 1985; Bandyopadyay *et al*., 1990; Bukhari, 1997a, 1997c) and *P. koelziana* (Zaeifi *et al.,* 2002).

Six ploidy values have been acquired for *P. cineraria*, five diploid and one tetraploid (Kumari *et al*., 1989).

Of the sixteen values recorded for *P. chilensis*, half were diploid and half tetraploid. Only one author records ploidy values other than 2*n* = 28 for this species. In two papers, Bukhari (1997b, 1997c) recorded ploidy values of 2*n* = 56 for *P. chilensis* in six seed accessions obtained from Sudan and in two from Kenya (Bukhari, 1997b, 1997c). In the same papers he recorded values of 2*n* = 28 for five seed accessions of *P. chilensis* from Chile. In a third paper, Bukahri (1997a) postulated that seed accessions of the so-called 'Sudan's Common Mesquite' obtained from Western, Central and Eastern Sudan and also from Kenya were of hybrid origin, and are possibly hybrids of *P. glandulosa* var. *torreyana* Benson and *P. chilensis* (Mol.) Stuntz emend. Burkart. It appears that the seed accession used in this paper (Bukhari, 1997a), obtained originally from Kenya, was identified in subsequent papers as *P. chilensis* (Bukhari, 1997b, 1997c). A small number of samples of Sudanese *Prosopis* examined at the Royal Botanic Gardens, Kew, which had been initially identified as *P. juliflora* had subsequently been re-classified and annotated as *P. chilensis*. These specimens did not match the botanical description of *P. chilensis* and were thought to be more probably *P. juliflora*, but they warrant closer investigation. Several researchers familiar with the genus have identified the common mesquite in Sudan as *P. juliflora* (Jackson, 1960; El Amin, 1990; El Fadl, 1997; Pasiecznik *et al*., 2001). All samples received for the present investigation from Sudan have been identified as *P. juliflora*, with ploidy values of 2*n* = 4*x* = 56. It is proposed, therefore, that the reports of tetraploidy in *P. chilensis* are erroneous and are in fact mis-identifications (Bukhari, 1997b, 1997c) which, if voucher specimens had been available for inspection, would have subsequently been correctly identified as *P. juliflora*.

Generally the ploidy of *P. glandulosa* is reported as 2*n* = 28 for the majority of counts, with ten of the twelve recorded being diploid. The two tetraploid values of 2*n* = 56 were obtained from two different samples collected from the same location, Kodai Road, Kodaikanal, Tamil Nadu, India, voucher number 29259 by Gill *et al*. (1984) and voucher number 29529 by Singhal *et al*. (1990). Singhal *et al.* (1990) considered that their meiotic count of $n = 28$ is the first report of polyploidy in this particular taxon. There is no information regarding the method used by Gill *et al*. (1984) but this also appears to be a meiotic count. It is possible that this is a mis-identification, perhaps due to the use of previous nomenclature, but this sample warrants closer inspection, to eliminate this possibility and to clarify whether or not *P. glandulosa* is indeed polyploid.

Most of the reports of polyploidy in this genus are recorded for *P. juliflora*, since in Table 4, 12 of the 21 values are tetraploid. None of the samples analysed by the authors of this study was diploid (Tables 1-3), suggesting that *P. juliflora* is entirely tetraploid. Looking more closely at ploidy values previously published for *P. juliflora* it is possible that at least some of these result from mis-identifications. *P. pallida* and *P. juliflora* are sympatric in parts of their natural and introduced ranges. They are particularly difficult to distinguish from one another and have frequently been confused, especially where introduced. Both species exist in Brazil, India and Senegal, indeed samples analysed in the present study originally identified as *P. juliflora* from Brazil (L095 & BRA 01) and Senegal (L096) have subsequently been shown to be *P. pallida*. Other Central American species can also be confused with *P. juliflora*, the sample of *P. juliflora* from Senegal, listed by Bukhari (1997b) as being diploid, is now listed as *P. glandulosa* in the DANIDA seed catalogue (DANIDAFSC, 2002). Bukhari also listed the same seed accession of *P. juliflora* from Mexico in his three papers as both diploid and tetraploid (Bukhari, 1997a, 1997b, 1997c). The ploidy of this particular sample is therefore not very clear. Reports of chromosome numbers with a base number other than $x = 14$ given by Sampath & Ramanathan (1949) and Kumari & Bir (1985) have already been considered, and these are also possible mis-identifications. One reference, which is generally considered as a mis-identification, is that of Bandyopadyay *et al.* (1990). The text of this paper refers to work carried out on samples of *P. chilensis*, whilst all figures and tables refer to samples of *P. juliflora*. Subsequent authors discussing this work have considered that the species analysed in this case was likely to have been *P. juliflora* rather than *P. chilensis*

(Bukhari, 1997a; Pasiecznik *et al*., 2001; Harris *et al*., 2003) and so this is included in the table as the former. If those ploidy values for this species are omitted which; (a) have been obtained from countries where identification has previously been a problem, or (b) where the ploidy value is not a multiple of the recognised base number for the genus, or (c) where more than one ploidy level has been reported for the same seed accession, or (d) where identification is otherwise unclear are excluded, only seven ploidy values remain, from four different references (Atchinson, 1951; Hunziker *et al.*, 1975; Bukhari, 1997a, 1997c). Six of these values are tetraploid and one diploid. Two of the six tetraploid values are reported from Pakistan (Bukhari, 1997a, 1997c), one from Cuba (Atchinson, 1951), and one each from Colombia, Haiti and Venezuela (Hunziker *et al.*, 1975). The single remaining diploid value is also from Colombia, reported by Hunziker *et al.* (1975). This paper reports polysomaty in this particular Colombian sample, in which 10% of 369 examined cells were diploid and the remainder tetraploid. Polysomaty appears to be quite common in chromosome counts made using the root tip squash method, and indeed was recorded for other species examined in this paper. However, no diploid cells were found in some samples of *P. juliflora* examined, for example samples from Haiti and a second Colombian sample. Hunziker *et al*. (1975) suggested that reports of polyploidy should be treated with caution, since if only few cells are examined these may simply be a small number of polyploid cells in an otherwise diploid tissue or plant. The authors considered that the sample from Colombia may be truly tetraploid in view of the large number of cells examined (Hunziker *et al.,* 1975).

In a recent paper, Zaeifi *et al.* (2002) recorded 30 ploidy values for three species of *Prosopis* in Iran; *P. cineraria*, *P. farcta* and *P. koelziana*. The chromosome number for *P. koelziana* was reported for the first time in their work. The majority of the 22 ploidy values recorded for this species were diploid with $2n = 28$ and the remaining three were tetraploid ($2n = 56$). The authors did not discuss polysomaty, although the root tip squash method was used and could it perhaps be expected to occur. They noted in their introduction that this species is newly-recognized, it is one of the least-known species and also appears to be an intermediate between *P. cineraria* and *P. farcta,* since there is a wide variation in the shape, habit and pod morphology of this species. They also noted that tetraploids have a greater number of longer pods in comparison with diploid populations.

In summary, most species whose ploidy has been examined appear to be diploid. Of those that have been reported to have both tetraploid and diploid individuals, reports of tetraploidy in only three species stand up to closer scrutiny, the species being *P. glandulosa*, *P. juliflora* and *P. koelziana.*

CONCLUSION

At present, ploidy values for 33 of the 44 species in the genus have been reported. Of these, 30 are diploid, and the remainder have variable ploidy, with both diploid and tetraploid samples of *P. glandulosa*, *P. juliflora*, and *P. koelziana* reported. Thus, the incidence of polyploidy in the genus is very low, especially when compared with its incidence in Leguminosae as a whole, which at 19% is twice as great as that for *Prosopis* (Hunziker *et al*. 1975). The results of the ploidy analysis show that of the nine species analyzed in this study all were diploid except one, *P. juliflora*. In this analysis, as in our previous one (Harris *et al*., 2003), all examined samples of *P. juliflora* were tetraploid. The conclusion of this analysis is that *P. juliflora* is an entirely tetraploid species and that the remaining eight species are diploid. However, a thorough and critical literature review does not entirely corroborate these findings, since other researchers report diploid samples of *P. juliflora* (Hunziker *et al*., 1975). In addition to this, whilst the results of flow cytometry analysis seem to suggest that the other species examined are diploid, including *P. glandulosa*, fewer samples of these species were examined, and tetraploid individuals of *P. glandulosa* and *P. koelziana* have been reported. This review corroborates the view of Burkart (1976) that the genus is mainly diploid, but our findings suggest that *P. juliflora* is normally tetraploid.

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Table 1. Ploidy of mature leaf samples of *Prosopis*. Identification of sample made by authors based on mature leaf characters of the individual from which leaves collected

Species	Sample	Collection site (if known)	Ploidy
*P. juliflora**	L112, L113	India, Dehli	4*x*
P. pallida	BRA 01	Brazil, Rio Grande del Norte, Natal	2*x*
*P. pallida**	L108, L109	India, Tamil Nadu, Mettupalayam	2*x*

*Samples previously published in Harris *et al.* (2003)

Table 2. Ploidy of seedling leaves of *Prosopis*. Identification made by authors based on mature leaves of the individual, either from which pods collected (LS) or of mature trees grown from the same seed accession (SL)

Species	Sample	Collection site (if known)	Ploidy	Sample type
P. chilensis	JOR P.CHIL	Jordan	2*x*	LS
	L093	Cape Verde Islands	2*x*	LS
P. juliflora	L031	Jordan, Wadi al-Mujib	4*x*	LS
	ANT 01	Antigua, Fitches Creek	4*x*	LS
	ANT 05	Antigua, Buckleys Main Road	4*x*	LS
	ANT 06	Antigua, Fort Road	4*x*	LS
	ANT 07	Antigua, McKinnons Lagoon	4*x*	LS
	ANT 09	Antigua, Shell Beach	4*x*	LS
	ANT 10	Antigua, Airport Road	4*x*	LS
	ANT 11	Antigua, Sunnyside School	4*x*	LS
	ANT 12	Antigua, Sugar Factory	4*x*	LS
	ETH 06	Ethiopia, Dire Dawa, Boreno	4*x*	LS
	ETH 08	Ethiopia, Afar, Dirk Kebele	4*x*	LS
	ETH 10	Ethiopia, Afar, Werer	4*x*	LS
	ETH 12	Ethiopia, Afar, Entiadoyta	4*x*	LS
	ETH 13	Ethiopia, Afar, Edelafafee	4*x*	LS
	GAL 01	Ecuador, Galapagos Islands	4*x*	LS
	GAL 02	Ecuador, Galapagos Islands	4*x*	LS
	JAM 01	Jamaica, St Catherines	4*x*	LS
	JOR 01, JOR 02	Jordan	4*x*	LS
	K~BURA 02	Kenya, Bura	4*x*	LS
	K~GARISSA 1	Kenya, Garissa	4*x*	LS
	K~HOLA RD 1, K~HOLA RD 4	Kenya, Hola Road	4*x*	LS
	K~HOLA RD 3	Kenya, Hola Road	4*x*	LS
	K~KAKUMA 1-2	Kenya, Kakuma	4*x*	LS
	K~KOLOKO 3	Kenya, Koloko	4*x*	LS
	K~LODWAR 1	Kenya, Lodwar	4*x*	LS
	K~MARIGAT 1-2	Kenya, Marigat	4*x*	LS
	NIG-01	Niger, Niamey, Grand Hotel	4*x*	LS
	NIG-02	Niger, Mardi, Soumarana	4*x*	LS
	OMAN 1	Oman, Salalah	4*x*	LS
	SL-01	Sri Lanka, Hambantota	4*x*	LS
	SL-02	Sri Lanka, Hambantota	4*x*	LS
	SUD 13	Sudan, Ed Debbra	4*x*	LS
	UAE 01, UAE 02, UAE 03, UAE 04, UAE 05, UAE 07, UAE 09, UAE 10, UAE 11, UAE 12	United Arab Emirates	4*x*	LS
	UAE 06, UAE 08	United Arab Emirates	4*x*	LS
	L025*	Cape Verde, Monte Vaca	4*x*	SL
	L027*	Cape Verde, Monte Vaca	4*x*	SL
	L029*, L030*, L033*	Jordan, Dead Sea	4*x*	LS
	L032*	Jordan, Wadi al-Mujib	4*x*	LS
	L037*	Jordan, Dead Sea	3*x*	LS
	L038*	India, Gujarat, Banni	4*x*	LS
	L041*	India, Gujarat, Tharad	4*x*	LS
	L047*	India, Rajasthan, Baburi	3*x*	LS

	L048*	India, Rajasthan, Beriganga	4*x*	LS
	L051*	India, Tamil Nadu, Mettupalayam	4*x*	LS
P. pallida	AUS 01, AUS 02, AUS 06, AUS 07, AUS 09, AUS 10, AUS 11, AUS 12, AUS 13	Australia, Queensland	2*x*	LS
	HAW 01	Hawaii, E.Maui, Lipoa, Kihei	2*x*	LS
	K~MOMB 5	Kenya, Mombasa	2*x*	LS
	L026*	Cape Verde, Monte Vaca	2*x*	SL
	L106*, L107*	India, Rajasthan, Jodhpur	2*x*	SL
Prosopis sp.	JOR PROS	Jordan	2*x*	LS

*Samples previously published in Harris *et al.* (2003)

Table 3. Ploidy of seedling leaves of *Prosopis*. Identification given by supplier of seed or plant material

Species	Sample	Collection site (if known)	Ploidy
P. affinis	DAN 01653/86	Peru, Codigo C1	2x
P. articulata		Living collection HDRA	2x
P. caldenia		Living collection HDRA	2x
P. chilensis	DAN 01591/86	Chile, Pama Alto-Bajo, Por rio Pama	2x
	L098	Chile, Chacabuco	2x
P. chilensis A		Living collection HDRA	2x
P. chilensis B		Living collection HDRA	2x
P. cineraria	CIN	India	3x
		Living collection HDRA	2x
P. glandulosa		Living collection HDRA	2x
	DAN 01211/83	Mexico, Concepcion del Oro, Zatetecas	2x
	DAN 01213/83	Mexico, Monterrey, Nuevo Leon	2x
	L088		2x
P. glandulosa var. *torreyana*	L103	USA, California	2x
P. juliflora	L110, L111	India, Jodhpur	4x
	L066*	Cape Verde, Pedregal	4x
	L075*	Cape Verde, Pedregal	3x
	L076*	Cape Verde, Pedregal	4x
	L078*	India, Uttar Pradesh, Lucknow	4x
	L080*, L082*, L084*	Commercial seed supplier	4x
	L083*	Commercial seed supplier	3x
P. laevigata	L102	Mexico	2x
P. lampa	DAN 01595/86	Chile, Atacama, Atacama	2x
P. pallida	DAN 01336/84	Peru, Huayruri, Santa Cruz	2x
	DAN 01351/84	Peru, Piura, Piura	2x
	DAN 01490/85	Peru, San Jacinto de Cachiche	2x
	DAN 01622/86	Peru, Jumana	2x
	DAN 01668/86	Peru, Codigo U2, Univ de Pinia	2x
	L074*	Cape Verde, Pedregal	2x
	L077*	Cape Verde, Monte Vaca	2x
	L072*	Peru, Trujillo (PF0442)	2x
	L087*	Peru, Trujillo (PF0428)	2x
	L090*	Peru, Trujillo (PF0446)	2x
	L095*	Brazil	2x
	L096*	Senegal, Richard Toll	2x
	L104*	Brazil, Pernambuco, Serra Tallada	2x
	L105*	Brazil, Pernambuco, Petrolina	2x
	MAU* 01	Mauritania, Aleg	2x
P. velutina	L101	USA, Arizona	2x
		Living collection HDRA	2x
Prosopis sp.	DAN 01281/84	Mexico, San Ignacio	2x

*Samples previously published in Harris *et al.* (2003)
Samples prefixed with DAN, kindly supplied by DANIDA FSC

Table 4: Previously-published ploidy values for *Prosopis*. Values in brackets show higher ploidy levels associated with polysomaty

Species	Ploidy		Origin	Primary reference. (values determined by)	Secondary References (lists or databases)	Method
	n	2*n*				
P. affinis Spreng.		28 (56, 112)	Argentina, Mendoza	Hunziker *et al.* (1975)	1	RTS
(as *P. affinis*)	14	28	Argentina, Entre Rios, Parana, Berduc	Naranjo *et al.* (1984)	2, 3	PMC
(as *P. algarobilla* Griseb.)		28(56)	Argentina, Entre Rios, Federacion, Los Conquitadores	Hunziker *et al.* (1975)	1, 4	RTS
(as *P. algarobilla* Griseb.)	14		Argentina, Entre Rios, Federacion, Federacion	Hunziker *et al.* (1975)	4	PMC
P. africana (Gull. & Perr.) Taub.		28	Senegal	Bukhari (1997a)		FC
		28	Senegal	Bukhari (1997c)	2	RTS
		28	Sudan	Bukhari (1997a, 1997c)	2	FC/RTS
P. alba Griseb.		28	Chile	Bukhari (1997b)	5, 6	FC
		28	Chile	Bukhari (1997c)	2	RTS
		28	Argentina, Cordoba, Capital Rio Primero	Hunziker *et al.* (1975)	1, 4	RTS
	14		Argentina, Formosa, Patino, Estancia La Primavera	Hunziker *et al.* (1975)	4	PMC
		28	Argentina, Entre Rios, Parana, Berduc	Naranjo *et al.* (1984)		RTS
	14	28	Argentina, Cordoba	Covas & Schnack (1947)	1, 7, 8	RTS
P. alpataco Phil.		28	Argentina, Las Heras, Camino a Villavicencio	Cherubini (1954)	7	RTS
	14		Argentina, La Pampa, Toay, Santa Rosa	Hunziker *et al.* (1975)	1, 4	PMC
		28 (56)	Argentina, Mendoza, Las Heras, Dique Papagallos	Covas & Schnack (1947)	7, 8	RTS
P. argentina Burkart		28 (56)	Argentina, Santa Rosa, Las Catitas	Cherubini (1954)	7, 9	RTS
		28 (56)	Argentina, Catamarca, Tinogasta, Copacobana	Hunziker *et al.* (1975)	4	RTS
		28	Prov. San Juan, Entre El Balde y Adan Quiroga	Covas & Schnack (1946)	1, 7, 8	RTS
P. caldenia Burk.		28 (56)	Argentina, La Paz, Paso de Tropsa	Cherubini (1954)	7, 9	RTS
P. campestris Griseb.		28	Cordoba	Covas & Schnack (1947)	7, 8	RTS
P. chilensis (Mol.) Stuntz		28	Chile	Bukhari (1997a)		FC
		28 (56)	La Rioja	Covas & Schnack (1947)	7, 8	RTS
		28 (56)	Argentina, Las Heras-Lavalle, Ramblon	Cherubini (1954)	7, 9	RTS
		56	W. Sudan	Bukhari (1997b)	5, 6	FC
		56	E. Sudan	Bukhari (1997b)	5, 6	FC

		56	Kenya	Bukhari (1997b)	5, 6	FC
		56	C. Sudan	Bukhari (1997b)	5, 6	FC
		28	Chile	Bukhari (1997b)	5, 6	FC
		56	W. Sudan	Bukhari (1997c)	2	RTS
		56	E. Sudan	Bukhari (1997c)	2	RTS
		56	C. Sudan	Bukhari (1997c)	2	RTS
		56	Kenya	Bukhari (1997c)	2	RTS
		28	Chile	Bukhari (1997c)	2	RTS
(as *P. siliquastrum* (Cav.) DC)		28	Chile	Bukhari (1997b)	5, 6	FC
(as *P. siliquastrum* (Cav.) DC)		28	Chile	Bukhari (1997c)	2	RTS
(as *P. siliquastrum* (Cav.) DC)		28 (56)	Argentina, Maipu, Pedregal	Cherubini (1954)	7, 9	RTS
P. cineraria (L.) Druce		28	Pakistan	Bukhari (1997c)	2	RTS
		28	Yemen	Bukhari (1997c)	2	RTS
	26		India, Punjab, Patiala, University campus	Kumari *et al.* (1989)	2	N/A
		28	Iran, Hormozgan, Bandar Lengeh, Maragh village	Zaeifi *et al.* (2002)		RTS
		28	Iran, Hormozgan, between Bandar Lengeh and Gavbani	Zaeifi *et al.* (2002)		RTS
		28	Iran, Hormozgan, Gavbani	Zaeifi *et al.* (2002)		RTS
P. denudans Benth.		28	Argentina, Rio Negro, Meseta de Somuncura, El Rincon	Hunziker *et al*. (1975)	1, 4	RTS
(as *P. patagonica* Speg.)		28 (56)	Argentina, Rio Negro, Valcheta, Sa. Grande	Hunziker *et al*. (1975)		RTS
P. farcta (Sol. ex Russell) J.F. Macbr.		28 (56)	Iran, Teheran	Cherubini (1954)	1, 7, 9	RTS
	14	28	Cyprus, Famagusta Bay	Oberpreiler & Vogt (1996)	2	N/A
		28	Iran, Yazd, Yazd	Zaeifi *et al.* (2002)		RTS
		28	Iran, Khuzestan, 165km to Ahvaz to Bushehr	Zaeifi *et al.* (2002)		RTS
		28	Iran, Khuzestan, 60km from Shoush to Ahvaz	Zaeifi *et al.* (2002)		RTS
		28	Iran, Khuzestan, 45km from Shoushtar to Ahvaz	Zaeifi *et al.* (2002)		RTS
		28	Iran, Khuzestan, Ramhormoz	Zaeifi *et al.* (2002)		RTS
P. ferox Griseb.		28 (56, 112)	Argentina, Jujuy, Quebrada de Humahuaca	Cherubini (1954)	7, 9	RTS
		28	Argentina, Jujuy, Quebrada de Humahuaca	Covas & Schnack (1947)	1, 7, 8	RTS
P. flexuosa DC	14	28 (56)		Hunziker *et al*. (1975)	1	RTS/PMC
		28	Chile	Bukhari (1997c)	2	FC

	14		Argentina, La Pampa, Utracan, Grai	Hunziker *et al*. (1975)	1, 4	PMC
	14		Argentina, Catamarca, Capayan, San Martin/El Medano	Hunziker *et al*. (1975)	1	PMC
		28 (56)	Argentina, Santa Rosa, Las Catitas	Cherubini (1954)	7, 9	RTS
		28	Chile	Bukhari (1997b)	5, 6	FC
P. glandulosa Torr.		28	Mexico	Bukhari (1997a)		FC
		26		Ramanathan (1950)	7	RTS
		28		Baquar *et al* . (1966)	1, 7	N/A
	14		Pakistan, Indus delta	Hunziker *et al*. (1975)	1	RTS
		28	Mexico	Bukhari (1997b)	5, 6	FC
	28		India, Tamil Nadu, Kodai Road	Gill *et al.* (1984)	3	N/A
(as *P. glandulosa* Torr. var. *torreyana*)		28	Mexico	Bukhari (1997c)	2	RTS
(as *P. glandulosa* Torr. var. *torreyana*)	28			Singhal *et al.* (1990)	2	PMC
(as *P. glandulosa* var. *glandulosa*)	14		USA, Texas	Hunziker *et al*. (1975)	1	PMC
(as *P. glandulosa* var. *torreyana*)		28	USA, California (cult.)	Hunziker *et al*. (1975)	1	RTS
(as *P. juliflora* DC var. *glandulosa*)		28 (56, 112)	USA, Texas, Spin	Cherubini (1954)	9	RTS
(as *P. juliflora* DC var. *torreyana*)		28 (56)	USA, Calif., Rancho Santa Ana Botanic Garden	Cherubini (1954)	9	RTS
P. hassleri Harms		28 (56)	Argentina, Formosa, Patino, Estancia La Primavera	Hunziker *et al*. (1975)	1, 4	RTS
P. humilis Gillies ex Hook.		28 (56, 112)	Argentina, Cordoba, Capital Rio Primero	Hunziker *et al*. (1975)	1	RTS
		28 (56, 112)	Argentina, Cordoba	Cherubini (1954)	7, 9	RTS
P. juliflora (Sw) DC		56	Cuba	Atchinson (1951)	7, 8	RTS
		52	India	Ohri (1996) in Bennett & Leitch 2004	6, 10	N/A
		28	Brazil, Recife, UFPE campus	Carvalheira *et al.* (1991)	2	RTS
		28	Senegal	Bukhari (1997b)	5, 6	FC
		28	Mexico	Bukhari (1997b)	5	FC
		28	Senegal	Bukhari (1997c)	2	RTS
		56	Mexico	Bukhari (1997c)	2	RTS
		56	Pakistan	Bukhari (1997c)	2	RTS
		52	India, Theri nr. Patiala	Kumari & Bir (1985)	3	RTS
	14	28 (56, 112)	Colombia, Bolivar, Cartagena, la Popa	Hunziker *et al*. (1975)	1, 4	RTS/PMC
		28 (56)	Brazil, Rio Grande do Norte, Mossoro, cult.	Hunziker *et al*. (1975)	1, 4	RTS

		56 (112)	Colombia, Tolima, Honda	Hunziker *et al*. (1975)	1, 4	RTS
		56 (112)	Haiti, de L'Oueste, Source Matlas/Port au Prince	Hunziker *et al*. (1975)	1, 4	RTS
		56 (112)	Venezuela, Lara, Barquisimeto, Quibor	Hunziker *et al*. (1975)	1, 4	RTS
	13		India, Kharar (near Chandigarh)	Bir & Sidhu (1967)	11	PMC
		52	India	Sampath & Ramanathan (1949)	7, 8	RTS
		56	Pakistan	Bukhari (1997a)		FC
		28	Senegal	Bukhari (1997a)		FC
		56	Mexico	Bukhari (1997a)		FC
(as *P. chilensis*)		56	India	Bandyopadyay *et al.* (1990)		RTS
P. koelziana Burkart		28	Iran, Baluchistan, Iranshahr, Bampour	Zaeifi *et al.* (2002)		RTS
		28	Iran, Baluchistan, Iranshahr, Bampour, Touran	Zaeifi *et al.* (2002)		RTS
		28	Iran, Baluchistan, Iranshahr	Zaeifi *et al.* (2002)		RTS
		28	Iran, Baluchistan, between Rigan & Bazman	Zaeifi *et al.* (2002)		RTS
		56	Iran, Kerman, Shahdad	Zaeifi *et al.* (2002)		RTS
		28	Iran, Kerman, 32km Bam to Zahedan	Zaeifi *et al.* (2002)		RTS
		28	Iran, Kerman, 27km Bam to Zahedan (Vakil abad)	Zaeifi *et al.* (2002)		RTS
		28	Iran, Kerman, Bam	Zaeifi *et al.* (2002)		RTS
		56	Iran, Hormozgan, between Bandar Lengeh and Gavbani	Zaeifi *et al.* (2002)		RTS
		28	Iran, Hormozgan, Hajiabad, Madanuyeh	Zaeifi *et al.* (2002)		RTS
		28	Iran, Hormozgan, Hajiabad, Tezerj	Zaeifi *et al.* (2002)		RTS
		28	Iran, Hormozgan, Hajiabad, Gahkom	Zaeifi *et al.* (2002)		RTS
		28	Iran, Hormozgan, Bandar e Kamir	Zaeifi *et al.* (2002)		RTS
		28	Iran, Hormozgan, Bandar e Lengeh, 5km E of Buchir	Zaeifi *et al.* (2002)		RTS
		28	Iran, Hormozgan, Gavbandi, Behdeh	Zaeifi *et al.* (2002)		RTS
		28	Iran, Hormozgan, Gavbandi	Zaeifi *et al.* (2002)		RTS
		56	Iran, Bushehr, Dashti, Razmabad	Zaeifi *et al.* (2002)		RTS
		28	Iran, Bushehr, Borazjan	Zaeifi *et al.* (2002)		RTS
		28	Iran, Khuzestan, Ramhormoz	Zaeifi *et al.* (2002)		RTS
		28	Iran, Khuzestan, Shoush	Zaeifi *et al.* (2002)		RTS
		28	Iran, Khuzestan, Shoush	Zaeifi *et al.* (2002)		RTS

		28	Iran, Khuzestan, 25km from Andimeshk to Shoush	Zaeifi *et al.* (2002)		RTS
P. kuntzei Harms ex Kuntze		28 (56, 112)	Argentina, Santago del Estero	Hunziker *et al.* (1975)	1	RTS
		28 (56, 112)	Argentina, Santiago del Estero, La Banda	Cherubini (1954)	7, 9	RTS
P. laevigata (Humb. & Bonpl ex Willd.) M.C. Johnst.		28	Mexico, Hidalgo, nr Meztitlan	Hunziker *et al*. (1975)	1, 4	RTS
		28	Mexico, Hidalgo, Santiago de Anaya	Tapia-Pastrana & Mercado-Ruaro (2001)		RTS/PMC
P. nigra (Griseb.) Hieron.	14		Argentina, Formosa, Patino, Estancia La Primavera	Hunziker *et al*. (1975)	1	PMC
	14		Argentina, Entre Rios, Gualeguaychu, Ceibas	Hunziker *et al*. (1975)	1, 4	PMC
		28 (56, 112)	Argentina, Entre Rios, Diamante, Puerto Diamante	Hunziker *et al*. (1975)	1, 4	RTS
	14		Argentina, Entre Rios, Parana, Berduc	Naranjo *et al.* (1984)	2, 3	PMC
P. pallida (Humb. & Bonpl. ex Willd.) Kunth		28	Peru	Bukhari (1997b)	5, 6	FC
		28	Peru	Bukhari (1997c)	2	FC
P. pubescens Benth.		28 (56)	USA, Calif., Rancho Santa Ana Botanic Garden	Cherubini (1954)	1, 7, 9	RTS
		28	Chile	Bukhari (1997a)		FC
		28	Chile	Bukhari (1997c)	2	FC
P. reptans Benth.		28 (56, 112)	Argentina, La Paz, Paso de las Tropas	Cherubini (1954)	7, 9	RTS
P. ruscifolia Griseb.		28		Covas (1950)	7, 8, 9	RTS
	14		Argentina, Formosa, Patino, Estancia La Primavera	Hunziker *et al*. (1975)	1, 8	PMC
	14		Argentina, Formosa, Patino, Arroyo Monte Lindo	Hunziker *et al*. (1975)		PMC
P. ruizleali Burk.	14		Argentina, Catamarca	Hunziker *et al.* (1975)	1	PMC
		28 (56)	Malargue	Covas & Schnack (1947)	7, 8	RTS
		28 (56, 112)	Argentina, San Rafael, Malalhue	Cherubini (1954)	7, 9	RTS
P. sericantha Gillies ex Hook.		28 (56, 112)	Argentina, Mendoza	Hunziker *et al.* (1975)	1	RTS
		28 (56, 112)	Argentina, La Paz, Desaguadero	Cherubini (1954)	7, 9	RTS
P. strombulifera (Lam.) Benth.		28		Covas & Schnack (1947)	8	RTS
P. tamarugo F.Phil.		28	Chile, Tarapaca	Hunziker *et al*. (1975)	1, 4	RTS
P. torquata (Cav. ex Lag.) DC		28 (56)	Argentina, La Rioja, Nonogasta	Cherubini (1954)	1, 7, 9	RTS
P. velutina Wooton		28 (56)	USA, California (cult.)	Hunziker *et al*. (1975)	1	RTS
(as *P. juliflora* DC var. *velutina*)		28 (56)	USA, Calif., Rancho Santa Ana Botanic Garden	Cherubini (1954)	9	RTS
P. vinalillo Stuck.	14	28 (56)	Argentina, Formosa, Patino, Estancia La Primavera	Hunziker *et al*. (1975)	1	RTS/PMC

Prosopis sp.	28 (56)	Argentina, Las Heras-Lavalle, Ramblon	Cherubini (1954)	7	RTS
	28 (56)	Argentina, Las Heras-Lavalle, Paradero	Cherubini (1954)		RTS
	28 (56, 112)	Argentina, Santa Rosa, Campo de la Dormida	Cherubini (1954)		RTS
	56	C. Sudan	Bukhari (1997a)		FC
	56	E. Sudan	Bukhari (1997a)		FC
	56	W. Sudan	Bukhari (1997a)		FC
	56	Kenya	Bukhari (1997a)		FC
	28		Turner & Fearing unpub.	7	

Secondary references; (1) Hunziker *et al*. (1977), (2) Index of Plant Chromosome Number (IPCN), (3) Goldblatt (1988), (4) Goldblatt (1981), (5) Bennett *et al*. (2000), (6) Bennett & Leitch (2004), (7) Federov (1969), (8) Darlington & Wylie (1955), (9) Cherubini (1981), (10) Bennett & Leitch (1997), (11) Moore (1973) .

Methods used to determine ploidy; RTS = Root tip squash, PMC = Pollen mother cell squash, FC = Flow cytometry, N/A = No information available.