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RESEARCH ARTICLE

Evidence for two domestication events of hyacinth bean (*Lablab purpureus* (L.) Sweet): a comparative analysis of population genetic data

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Abstract Studying molecular genetic relationships can substantially contribute to the understanding of the pathways of domestication of a species. Although an increasing number of molecular genetic studies have been performed on *Lablab purpureus* (hyacinth bean), many covered germplasm of restricted geographic origin or limited intra-specific systematic position. Integrating the molecular diversity found with phenotypic or morpho-agronomic diversity is also deficient. This investigation combines findings of eight molecular genetic studies that include about 400 accessions of both wild and cultivated germplasm, thus providing the largest assessment of diversity in *Lablab purpureus* to date. In particular, results from a recent molecular investigation (Robotham and Chapman 2015) are revisited and reinterpreted by integrating them with known phenotypic diversity. Wild accessions clearly fall into two types, with characteristic pods—2-seeded and 4-seeded. The large majority of cultivated types are more closely related to 4-seeded pod-types. Certain cultivated 2-seeded pod-type accessions from Ethiopia are genetically closer to wild 2-seeded pod-types. These two major phenotypes are reflected in two chloroplast DNA haplotypes A and B. Hence, two domestication events appear to exist in *L. purpureus* based on this combined data. No other geographic patterns of diversity, which might assist to trace the dispersal of *L. purpureus*, were found as cultivated accessions predominantly fell into 2-3 major groups. In all studies, the greatest genetic diversity was found in Africa, making Ethiopia one of the probable centers of domestication.

Keywords Center of origin · *Dolichos lablab* · Genetic diversity · *Lablab purpureus* · Legume · Microsatellites · Underutilized crop

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Introduction

Analyzing genetic diversity through the application of molecular markers is becoming more and more common for underutilized and neglected crops, such as the hyacinth bean or lablab bean (*Lablab purpureus* (L.) Sweet). Different molecular markers have been applied as reviewed by Maass et al. (2010); however,

few studies have attempted to integrate the molecular diversity found with phenotypic or morpho-agronomic diversity (e.g., Maass et al. 2005; Rai et al. 2011; AbdAllah et al. 2015). Most research rather focused on interpreting diversity in relation to geographic provenance and/or sub-specific rank.

Several molecular genetic papers, although largely by coincidence, contain accessions from the proposed core collection (Pengelly and Maass 2001; Maass et al. 2005) that supposedly covers a large portion of known diversity with representative accessions. Venkatesha et al. (2007), Wang et al. (2007), Islam (2012) and Robotham and Chapman (2015) are the only broad studies on lablab found so far that employ Simple Sequence Repeats (SSR), beside a few investigations with very limited coverage of local germplasm from Kenya (Shivachi et al. 2013), India (Saravanan et al. 2013) and China/Kenya (Zhang et al. 2013), so their results cannot easily be linked to others. While Venkatesha et al. (2007) use SSR markers developed for soybean (*Glycine max* (L.) Merr.), barrel medic (*Medicago truncatula* Gaertn.), beans (*Phaseolus* L. spp.) and *Vigna* Savi spp., Wang et al. (2007) and Islam (2012) employ the same markers derived from soybean, barrel medic and cowpea (*Vigna unguiculata* (L.) Walp.). For the first time, Robotham and Chapman (2015) used SSR markers specifically designed from the *Lablab* transcriptome (Chapman 2015). They found that genetic variation was highest in eastern African accessions, and that cultivated lines from East Africa were more closely related to the wild subspecies, *L. purpureus* subsp. *uncinatus* Verdc., summarizing an East African origin and subsequent dispersal.

The current paper attempts to provide an overall assessment of diversity patterns in *Lablab purpureus* by reanalyzing and interpreting Robotham and Chapman's (2015) results in depth and connecting key findings with other molecular genetic studies in order to improve our understanding on pathways of domestication of this multipurpose legume.

Methodology

Reanalysis of Robotham and Chapman (2015)

Robotham and Chapman (2015) obtained a total of 91 geographically diverse *L. purpureus* accessions from the USDA National Plant Germplasm System (http://www.ars-grin.gov/npgs/index.html) and the Forage Germplasm Collection of the International Livestock Research Institute (ILRI) (Robotham and Chapman 2016). Provenances of the accessions have been described by Robotham and Chapman (2015, Online Resource 1; 2016), however, all 8 accessions previously labelled 'ex Australia' were from Kenya, except for one (PI 401553) from India. Highly likely, all subsp. *bengalensis* (Jacq.) Verdc. accessions were correctly identified, while those belonging to other subspecies might not, due to different taxonomic concepts. One accession (PI 532672) supplied by USDA as subsp. *uncinatus* Verdc. has been discarded from the reanalysis as it was identified instead as *Dolichos sericeus* E.Meyer (A Moteetee, pers. comm.). The 90 accessions were characterized by five SSR loci, and data are reanalyzed here to investigate relationships among accessions and identify genetic groups. The program GenAlEx (Peakall and Smouse 2006) was applied for principal coordinate analysis (PCoA). Robotham and Chapman (2015) describe further details on data analysis. Finally, Fig. 2 of Robotham and Chapman (2015) was reproduced and redrawn with additional details for in-depth interpretation.

Comparative analysis of molecular studies of Lablab

Accessions common in various studies were identified by revising passport data made available by the Commonwealth Scientific and Industrial Research Organisation (CSIRO), ILRI, USDA (https://npgsweb.ars-grin.gov/gringlobal/search.aspx), the World Vegetable Center (AVRDC, http://203.64.245.173/search.asp) and the International Center for Tropical Agriculture (CIAT). Further molecular genetic studies examined for shared germplasm were Liu (1996), Sultana et al. (2000), Maass et al. (2005), Wang et al. (2007), Maass and Tefera (2008), Islam (2012) and Venkatesha et al. (2007) – although the latter had already been revised and further interpreted by Maass et al. (2010; Fig. 1). The number of primers/primer pairs applied in these studies ranged from 3 to 48 (Table 1). In two studies (Liu 1996; Wang et al. 2007), erroneous seed material appears to have been used that could lead to confusing results.[1] Although Wang

[1] Seed mass (22.0 g/100 seeds) of the seed used from accession CPI 60216 by Liu (1996) is too high for a wild accession. Also, this accession is hidden among the cultivated ones in Liu's

et al. (2007) did not discriminate it explicitly, accession PI 280861 has to be a wild type due to its low seed mass (7.53 g/100 seeds); the corresponding Australian accession (CPI 28701) has been characterized as a wild 4-seeded pod-type by BC Pengelly in Australia (unpublished).

All eight revised investigations contained both wild and cultivated germplasm accessions, except for Islam (2012). While the investigation by Maass et al. (2005) comprised the largest number of wild accessions (N = 10), that by Robotham and Chapman (2015) was the first of its kind with a substantial number of cultivated 2-seeded pod-types from Ethiopia (N = 6). The eight studies considered included a total of about 400 apparently different accessions (Online Resource 1) with relatively little overlap (Table 1). However, a few accessions have been used almost in all studies, such as CPI 34777 (syn. ILRI 11617/PI 388003) and CPI 52508 (syn. ILRI 21049). The largest overlap with Robotham and Chapman's (2015) research was with 17 and 15 accessions in Maass et al. (2005) and Wang et al. (2007), respectively (Table 1); and 14 accessions were contained from the core collection (Table 1) suggested by Pengelly and Maass (2001) and Maass et al. (2005) (Online Resource 2).

Results

Genetic relationships

This is the largest assessment of diversity conducted in *Lablab purpureus* including both wild and cultivated germplasm, combining findings of eight molecular genetic studies (Table 1). From all studies it is clear that the large majority of accessions seems to be genetically fairly similar and that the molecular markers provide limited resolution for the bulk of the cultivated accessions. In almost all studies, there are 2 or 3 major clusters containing essentially all cultivated germplasm (Table 2), with little correspondence to the provenance of the accessions. Wild accessions, in contrast, usually fall into various small groups despite the number of accessions being much fewer. Except for Venkatesha et al. (2007), in all studies wild and cultivated accessions fell broadly into different groups (Table 2); and Sultana et al. (2000) identified two RAPD markers that discriminate between wild and cultivated accessions.

When revisiting the research by Robotham and Chapman (2015), it was apparent that particularly some Ethiopian cultivated accessions clustered with other wild African accessions, thus, forming a distinct group (dashed line; Fig. 1). All accessions of this Cluster 2 have a characteristic pod shape, typically containing 2 seeds. This pattern, with the 2-seeded or 4-seeded pod-types being genetically distinct is also evident in the studies revisited that contained accessions from the two wild types (Liu 1996; Maass et al. 2005; Maass and Tefera 2008) (Table 2). Just three studies (Maass et al. 2005; Maass and Tefera 2008; Robotham and Chapman 2015) included accessions of the Ethiopian cultivated 2-seeded pod-type; they clearly fell into groups distinct from the bulk of the cultivated accessions (Fig. 1). The PCoA did not reveal other groups.

Chloroplast DNA haplotypes

Based on a single polymorphism found at chloroplast locus *psb*M2-*trn*D_GUC (a G/C single nucleotide polymorphism), the *L. purpureus* germplasm studied diverged into two clear groups that Robotham and Chapman (2015) named haplotype A and B (G and C nucleotide, respectively). The large majority of accessions was scored haplotype B, while only 10 were haplotype A.

All haplotype A belonged to the 2-seeded pod-type both wild and cultivated, except for accession PI 280881 (syn. CPI 28701) from Kenya, a 4-seeded pod-type (BC Pengelly, unpublished). Cultivated 2-seeded pod-types were from Ethiopia, while the wild accessions with haplotype A were from Zambia and

Footnote 1 continued
Fig. 2; as a wild 4-seeded pod-type it should be relatively close to wild accession CPI 31113. In other publications, it doubtlessly seems wild (Sultana et al. 2000; Maass et al. 2005).

Seed mass (2.37 g/100 seeds) of the seed used from accession PI 532672 (Swaziland) by Wang et al. (2007) is far too low for *L. purpureus*; also Fig. 3 (Wang et al. 2007) displays seed untypical for *Lablab* due to its shape and as the typical white aril is missing. A Moteetee (pers. comm.) suggests it belongs to *Dolichos sericeus* E. Meyer. Robotham and Chapman (2015) scored it as haplotype B; otherwise it was inconspicuous for the molecular analysis, while morphologically the seed resembles that of Wang et al. (2007).

Accession PI 532638 (Zimbabwe) in Wang et al. (2007) might not belong to *L. purpureus* due to its low seed mass (5.37 g/100 seeds).

Table 1 Characteristics of eight comprehensive *Lablab purpureus* germplasm collections studied by different molecular markers and overlap (common accessions) with the study by Robotham and Chapman (2015)

Study—molecular marker applied	Germplasm characteristics[a]	Common accessions (no.)[a]	Core collection accessions included (ID)	
			Wild[b]	Cultivated[b]
Liu (1996)—48 RAPD primers (N = 40)	Comprehensive CSIRO collection: India, E + S Africa	9	CPI 51564*, 60216, 69498*	CPI 30702, 34777, 67639, 41222, 52508, 76996, 76998, 100602, 106471, cvs. Rongai, Highworth;
Sultana et al. (2000)—11 RAPD primers (N = 102)	Broad S, SE + E Asian (72) collection, 'exotic' accessions from CSIRO (30)	9	CPI 51564*, 52437, 60216	CPI 29398, 30702, 34777, 36903, 41222, 52508, 52535, 52544, 76998, 81626, cvs. Rongai, Highworth;
Maass et al. (2005)—4 AFLP primer pairs (N = 102)	Comprehensive CSIRO (98) collection, few ILRI accessions	17	CPI 51564*, 60216, 69498*; CPI 106585#	CPI 29398, 34777, 35894, 36903, 41222, 52508, 52511, 52535, 52544, 76996, 76998, 81626, 99985, 100602, 105036, 106471,106494, 106500, 106548, CQ2975, cv. Rongai; ILRI 13689
Venkatesha et al. (2007)—3 AFLP primer pairs (N = 78)	Broad S Indian (67) collection, some African accessions from CSIRO and ILRI	5	CPI 51564*, 60216	CPI 36903, 41222, 52508, 52535, 52544, 67639, cvs. Rongai, Highworth; ILRI 6536, 6930, 13700
Wang et al. (2007)—26 SSR primer pairs (N = 45)[c]	Comprehensive USDA collection: China, SE Asia, India, E + S Africa, Europe, Americas	15	not applicable	CPI 34777, 35894, 36903
Maass and Tefera (2008)—4 AFLP primer pairs (N = 40)	Diverse collection from CSIRO + ILRI, mainly from core collection, few Indian feral and Tanzanian accessions	9	African accessions not applicable; CPI 106585#	CPI 29398, 34777, 35894, 36903, 52508, 52535, 52544, 67639, 76996, 76998, 81626, 96924. 99985, 100602, 106471, 106548, CQ2975, cv. Highworth; ILRI 6930, 11613, 11615, 11632, 13694, 13695, 13700, 14411, 14437, 14442
Islam (2012)—13 SSR primer pairs (N = 65)	Broad Bangladesh collection (42), SE Asian accessions from AVRDC, core accessions from CSIRO + ILRI	3	not applicable	CPI 34777, 35894, 52508, 76996, 81626, 100602, 106548; ILRI 14437
Robotham and Chapman (2015)—5 SSR primer pairs (N = 90)[c]	Comprehensive collections from USDA (29) and ILRI (61)	not applicable	CPI 51564*, 69498*	CPI 34777, 41222, 81626, 100602; ILRI 13687, 13692*, 13694*, 13695*, 13700, 13702*, 14411, cv. Highworth (as PI401553)

AFLP amplified fragment length polymorphism, *RAPD* random amplified polymorphic DNA, *SSR* simple sequence repeat markers

[a] Germplasm overlap of Robotham and Chapman (2015) with that of other studies; AVRDC, The World Vegetable Center; CSIRO, Commonwealth Scientific and Industrial Research Organisation; ILRI, International Livestock Research Institute; USDA, United States of America Department of Agriculture

[b] An asterisk (*) indicates 2-seeded pod-types; a hash (#) is for Indian feral accessions; not applicable, not available

[c] Numbers, after discarding data from one accession (PI 532672) that did not belong to *Lablab purpureus*

Zimbabwe. Accession ILRI 18633 (Malawi) was also scored haplotype A and had, therefore, initially been wrongly assigned to subsp. *purpureus* by Robotham and Chapman (2015) because it was a wild 2-seeded pod-type (Wiedow 2001). The other way round, all 2-seeded pod-types fell under haplotype A except for accession ILRI 13702 from Ethiopia; this might have been caused by erroneous seed handling or accidental crosses. Although accession ILRI 13694 failed to sequence and was recorded as missing data when

Table 2 Major findings of diversity patterns in eight comprehensive *Lablab purpureus* germplasm collections studied by different molecular markers

Study—molecular marker applied	Major findings	2-seeded versus 4-seeded pod-types	Cluster groups (no.)[a]	
			Wild	Cultivated
Liu (1996)—48 RAPD primers (N = 40)	Clear distinction between wild and cultivated types; diversity rather low, except for wild types; great majority of cultivated belong to 1 group; African and Asian accessions integrate thoroughly	Clear distinction between the two wild types	2 (5)	4 (35)
Sultana et al. (2000)—11 RAPD primers (N = 102)[b]	Clear distinction between wild and cultivated types; cultivated form 2 major groups; no relation to geographical provenance	Certain divergence between wild types, but not clear (Only one accession 2-seeded type)	5 (8)	2 (93)
Maass et al. (2005)—4 AFLP primer pairs (N = 103)[b]	Clear distinction between wild and cultivated types; cultivated form 2 major groups; neither relation to geographical provenance nor subsp	Clear divergence both in wild and cultivated (ETH) types	5 (10)	7 (91)
Venkatesha et al. (2007)—3 AFLP primer pairs (N = 78)	S-Indian accessions all very similar; large diversity in African germplasm; wild accessions not distinct from cultivated African	Distinction not clear (Only one accession 2-seeded type)	n.d.	n.d.
Wang et al. (2007)—26 SSR primer pairs (N = 45)	Distinction between wild and cultivated types; cultivated form only 1 group; no relation to geographical provenance	n.a. (Only 4-seeded types included?)	1 (1)	1 (44)
Maass and Tefera (2008)—4 AFLP primer pairs (N = 40)[b]	Clear distinction between wild and cultivated types; neither relation to geographical provenance nor subsp	Clear divergence both in wild and cultivated (ETH) types	2 (2)	2 (33)
Islam (2012)—13 SSR primer pairs (N = 65)	Little relation to geographic provenance, none to subsp.; relatively low genetic diversity as no wild types included	n.a. (Only 4-seeded types included?)	n.a.	8 (65)
Robotham and Chapman (2015)—5 SSR primer pairs (N = 90)	Some distinction between wild and cultivated types; cultivated form 3 major groups; neither relation to geographic provenance nor subsp	Strong divergence both in wild and cultivated types	5 overall	

AFLP amplified fragment length polymorphism, *RAPD* random amplified polymorphic DNA, *SSR* simple sequence repeat markers, *ETH* Ethiopia

[a] Number of accessions in brackets; n.a., not available; n.d., not determined

[b] Sultana et al. (2000), Maass et al. (2005) and Maass and Tefera (2008) contain 1, 2 and 5 Indian 'wild' accessions, respectively, considered feral escapes from domestication, neither here counted as wild nor as cultivated

scoring haplotypes (Robotham and Chapman 2015), it correctly clustered among the cultivated 2-seeded pod-type accessions from Ethiopia (Maass and Tefera 2008) (Cluster 2 in Fig. 1) and, hence, would have highly likely be scored haplotype A.

All other cultivated accessions were scored haplotype B. There was no distinction between subsp. *purpureus* and subsp. *bengalensis*. Wild 4-seeded pod-type accessions had not been identified previously in the germplasm studied by Robotham and Chapman (2015). Only two such accessions were included in this study: ILRI 14550 from Zimbabwe (Wiedow 2001) and PI 280881 (syn. CPI 28701) from Kenya (BC Pengelly, unpublished). The latter had failed to sequence (Robotham and Chapman 2015) and clustered unexpectedly with haplotype A accessions (Cluster 2 in Fig. 1). Accession ILRI 14550, on the other hand, grouped as expected with Cluster 1 in the SSR analysis (Fig. 1). Hence, these two wild 4-seeded accessions related inconsistently to the remainder of accessions studied by Robotham and Chapman (2015).

The clear divergence into two haplotypes of comprehensive germplasm studied by Robotham and

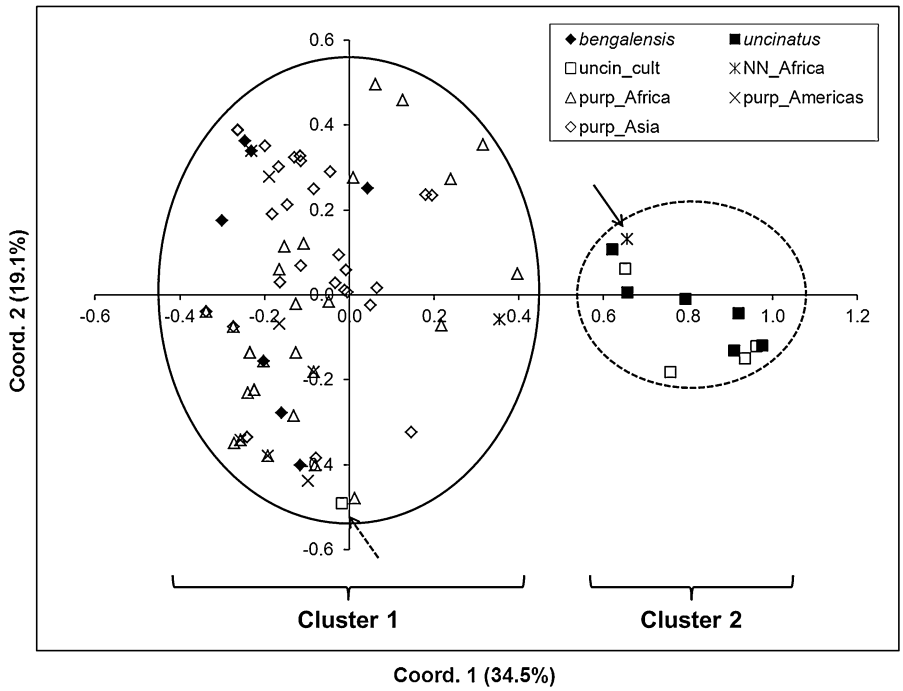

Fig. 1 Principal coordinate analysis (PCoA) of 90 accessions of *Lablab purpureus* assessed using five SSR loci (reanalyzed from Robotham and Chapman 2015). Accessions are coded according to subspecies [subsp. *uncinatus* (uncin), subsp. *purpureus* (purp) or subsp. *bengalensis*] and then by provenance. NN *nomen nominandum* refers to unresolved systematic treatment; cult, Ethiopian cultivated 2-seeded pod-types. Note the presence of the latter accessions with other wild African subsp. *uncinatus* provenances of the same morphological type under Cluster 2. A *solid arrow* indicates wild accession PI 280881 (syn. CPI 28701), a *dashed arrow* cultivated accession ILRI 13702 that both fall out of the patterns

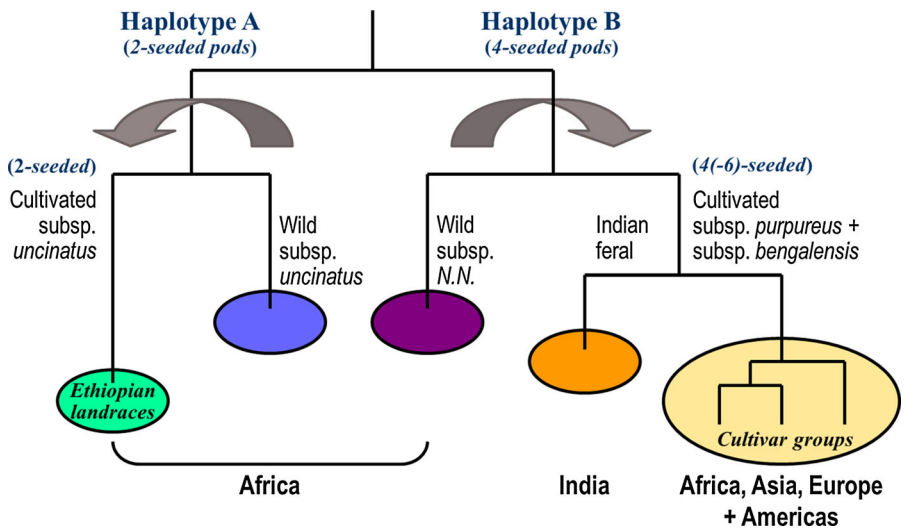

Fig. 2 Proposed relationships of two wild African subtaxa of *Lablab purpureus* with feral escapes from India and cultigens worldwide, reflecting two domestication events as indicated by *curved arrows* (modified from Maass 2016); *NN nomen nominandum* refers to unresolved systematic treatment

Chapman (2015) supports the hypothesis of two domestication events put forward by Maass and Tefera (2008) and Maass (2016) (Fig. 2). Unfortunately, their study contained too few accessions of the wild 4-seeded pod-type to corroborate the relationship of this wild with the cultivated 4(-6)-seeded pod-types.

Discussion

The understanding of genetic relationships depends to a great extent on the available set of germplasm accessions under investigation. An increasing number of molecular genetic studies have been carried out on *L. purpureus*, although many of them included germplasm of restricted geographic origin or limited intra-specific diversity. Regrettably, this limits the interpretation of many results in the context of worldwide distribution of genetic variation and the pathway to domestication. The eight studies revisited here all contained a certain, although variable, portion of the core collection proposed by Pengelly and Maass (2001) and Maass et al. (2005). Although unintentional to some extent, this overlap of accessions facilitated comparison and integration of results.

Center of origin and domestication

Findings by Robotham and Chapman (2015), interpreted in more depth here, support the previous view (Verdcourt 1970; Maass et al. 2005; Maass 2016) that the center of origin of *L. purpureus* is eastern and southern Africa. This is currently the only known region where wild forms occur naturally. Also, a higher level of genetic diversity is evident in African cultivated germplasm. Four of five molecular clusters resolved by Robotham and Chapman (2015) contain accessions from Ethiopia, a fact that supports this area to be considered a center of diversity and one of the most probable candidate areas of domestication (Maass 2016).

Despite the occurrence of wild forms only in Africa, domestication of *Lablab* might have taken place elsewhere. Millennia before archaeological finds in Africa, *Lablab* was located in archaeological sites in India alike other crops of African origin (Blench 2003). Due to their intermediate status in molecular studies, though, Indian 'wild' 4-seeded pod-type forms have been understood as feral escapes from domestication (Maass et al. 2005; Maass and Tefera 2008). Nevertheless, no evidence exists as yet that lablab has been trans-domesticated on the Indian sub-continent; instead this unquestionably constitutes a secondary center of diversity of the species (Maass 2016). Our hypothesis of *Lablab* originating in East Africa means that of the dozen or so legumes widely cultivated in South Asia (Fuller and Harvey 2006) only lablab and cowpea (Ng 1995; Ogunkanmi et al. 2005) are introduced species.

Genetic patterns of diversity corresponding to geographic provenance of accessions have not been identified notwithstanding the millennia of crop domestication and trans-continental dispersal. No patterns have been detected that show onward movement of *Lablab* first from Africa to India, later eastward to other Asian countries; nor are they evident from Africa to the Americas. *Lablab* was introduced to both Brazil and the Caribbean during the trans-Atlantic slave trade (Carney and Rosomoff 2009) so that certain genetic modification might have taken place over the past about 400 years when the crop was adapted to new environmental conditions, possibly leading to new genetic diversity. In all studies, the large majority of cultivated accessions fully integrate in a few main clusters regardless of their provenance, except for the 2-seeded pod-type from Ethiopia. This lack of differentiation of *Lablab* according to geographic region at putatively neutral molecular markers suggests a high level of gene flow between regions, which ultimately depends on seed exchange.

Attributes of diversity, taxonomy and domestication

Wild forms are characterized by greyish brown, relatively flat seeds of low weight and generally similar shape, whereas domesticated forms show ample variation in seed colour, size and shape (Maass and Usongo 2007). A more than tenfold seed mass increase from about 60 g (4-seeded pod-types) and 100-120 g (2-seeded pod-types) to more than 600 and up to 1000 g per 1000 seeds, respectively, has been recorded (Maass 2016). However, cultivated large 2-seeded pod-types with seed mass of over 600 g are only known from Ethiopia (Verdcourt 1970; Thulin

1989) and have received little attention in research. The great majority of cultivated accessions have about 200 to 400 g seed mass.[2]

Wild subsp. *uncinatus* is illustrated by Rivals (1953, p. 535 Pl. B-1) and described as normally having 2-seeded pods;[3] the illustration is based on plants from Ethiopia identified by A. Braun in 1841. The respective herbarium specimen (P: Schimper, No. 1779) instead shows a cultivated plant with larger pod size than described by Verdcourt (1970) for the wild subspecies, with the type collected from Kilifi, Kenya (isotype EA: Polhill RM & Paulo S, No. 699). Rivals (1953) designates another wild variant with 3-4-seeded pods as subsp. *crenatifructus,*[4] a taxon that Verdcourt (1970) rejected because no valid description existed according to the International Code of Botanical Nomenclature. This subsp. might be useful for improving the definition of wild 4-seeded pod-types (*nomen nominandum* in Figs. 1 and 2). The universal determination of all wild plants as subsp. *uncinatus* has caused confusion. Verdcourt's (1970) treatment in the Flora of Tropical East Africa was, consequently, too general by pooling all wild plant variants under subsp. *uncinatus* and should be revised making use of recent molecular understanding.

The cultivated 2-seeded pod-types do not correctly fall under what Verdcourt (1970) defines as subsp. *uncinatus* because of their size and cultivation status. The research by Robotham and Chapman (2015) contains the largest sample of this type ever investigated with molecular markers, demonstrating their genetic uniqueness by nuclear SSRs and scoring them all, except one, as haplotype A, the typical haplotype for wild 2-seeded accessions. Cultivated *L. purpureus* subsp. *bengalensis*, recognized by Verdcourt (1970), is less ambiguous in terms of its morphology. It is characterized by long, linear, kidney-bean-like pods and higher seed numbers per pod (up to 6, sometimes more) than typical subsp. *purpureus*; in addition, the long axis of mature seeds is placed parallel to the pod suture, in contrast to the perpendicular seed placement in subsp. *purpureus* (Rivals 1953). The seven accessions of subsp. *bengalensis*, however, were scattered in the PCoA and were distributed between two of the five clusters in the structure analysis (Robotham and Chapman 2015). This confirms similar findings by Maass et al. (2005), overall indicating this is not a genetically coherent group and more likely represents multiple (at least two) derivations from cultivated subsp. *purpureus*. In other studies, it was not indicated whether accessions belonged to subsp. *bengalensis*. Verdcourt (1970) suggests subsp. *purpureus* and subsp. *bengalensis* to interbreed freely.

Evidence for two domestication events

Results by Robotham and Chapman (2015) support the hypothesis of two domestication events put forward by Maass et al. (2005), Maass and Tefera (2008) and Maass (2016). There are two distinct origins for the domesticated species, one domesticated from wild plants with 2-seeded pods and slightly higher seed mass (100-120 g/1000 seeds) and one domesticated from wild plants with 4-seeded pods and lower seed mass (about 60 g/1000 seeds). These two wild types also have clearly distinct pod shapes. Robotham and Chapman (2015) score them as haplotype A and B, respectively, based on chloroplast DNA sequencing. Due to the low number of accessions from the wild 4-seeded pod-type, however, their explicit classification as one of the two haplotypes was not possible in this study. Other research, though, showed clear divergence between the two wild types (Table 2).

Regarding genetic relationships and domestication: wild plants have been successfully crossed with domesticated ones (Liu 1998a, b; Venkatesha 2012, cited in Bohra et al. 2014), using accessions CPI 24973 (2-seeded pod-type) by the former and CPI 31113 and CPI 60216 (4-seeded pod-type) by the latter; cultivated lines employed were cvs. Rongai and HA4, respectively. These two cultivars are typical haplotype B plants. It appears, thus, that both wild types are compatible in cross-breeding. However, knowledge about compatibility of haplotype A with B is lacking at larger scale and,

[2] Based on more than 380 germplasm accessions evaluated in Redland Bay, Australia (BC Pengelly unpublished) and Zwai, Ethiopia (BL Maass unpublished; C Wiedow 2001) and in Bangladesh (Islam 2008, 2012).

[3] Rivals (1953): "gousses déhiscentes, ordinairement dispermes, de petites dimensions (30 × 13 mm) élargies vers l'extrémité (Fig. 1). Graines petites noires (5 × 4 × 2,5 mm) presque plates sur les côtés."

[4] Rivals (1953): "Gousses de petite taille très déhiscentes atteignant leur plus grande largeur vers le milieu, renfermant 3 à 4 graines noires de petites dimensions".

particularly, involving the cultivated 2-seeded pod-type plants from Ethiopia.

Conclusions

The comparative analysis and integrated interpretation of eight independent molecular genetic studies of *L. purpureus* with relatively small overlap, particularly from a species core collection, comes to the following major conclusions:

No geographic diversity patterns were detected to help unravel dispersal from the center of origin, which is eastern and southern Africa, with Ethiopia as a center of diversity. Additional molecular markers may help reveal geographic patterns of diversity and dissemination pathways.

Two domestication events appear to exist in *L. purpureus* based on two discrete haplotypes A and B. While cultivated haplotype A is restricted to Ethiopia, haplotype B cultigens are found worldwide.

There is need for a taxonomic revision of wild *L. purpureus* beyond the current broad circumscription of subsp. *uncinatus* (sensu Verdcourt 1970).

Future researchers are recommended not to analyze data according to presumed subspecies as most germplasm collections do not seem to have good taxonomic identification, and systematic treatment has not kept up with molecular understanding.

This comparative analysis demonstrates the virtue of defining and using a species core collection in order to compare findings among different collections and derive a broader view of diversity patterns.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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