


Review

Perennial Grain Legume Domestication Phase I: Criteria for Candidate Species Selection

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Abstract: Annual cereal and legume grain production is dependent on inorganic nitrogen (N) and other fertilizers inputs to resupply nutrients lost as harvested grain, via soil erosion/runoff, and by other natural or anthropogenic causes. Temperate-adapted perennial grain legumes, though currently non-existent, might be uniquely situated as crop plants able to provide relief from reliance on synthetic nitrogen while supplying stable yields of highly nutritious seeds in low-input agricultural ecosystems. As such, perennial grain legume breeding and domestication programs are being initiated at The Land Institute (Salina, KS, USA) and elsewhere. This review aims to facilitate the development of those programs by providing criteria for evaluating potential species and in choosing candidates most likely to be domesticated and adopted as herbaceous, perennial, temperate-adapted grain legumes. We outline specific morphological and ecophysiological traits that may influence each candidate's agronomic potential, the quality of its seeds and the ecosystem services it can provide. Finally, we suggest that perennial grain legume breeders and domesticators should consider how a candidate's reproductive biology, genome structure and availability of genetic resources will determine its ease of breeding and its domestication timeline.

Keywords: Fabaceae; perennial grain; domestication; ecosystem services; domestication pipeline; crop candidates

1. Introduction

The legume family (Fabaceae) is one of the largest families of flowering plants with more than 19,500 species [1,2] and an estimated 732–765 genera [3–5]. The well-known symbiotic relationship between legumes and root-nodule bacteria (hereafter, rhizobia) supplies biologically fixed nitrogen (BNF) to natural and agroecosystems around the globe [6]. BNF, which may be considered the most fundamentally important biological process on earth aside from photosynthesis [7], reduces more than 100 Tg dinitrogen to ammonia each year [8]. This form of nitrogen (N) is directly useable by legumes, and eventually, through nutrient cycling and consumption, becomes available to other plants and organisms. In fact, the entire nutritional N requirement for humans is obtained directly or indirectly from plants [9]. For this reason, legumes have long been exploited in agriculture as essential rotational species in cropping systems to improve soil fertility and increase annual cereal yields, and they continue to supply approximately 13% of the annual global agricultural N requirements (30–50 Tg) [10,11]. In addition to the direct benefits of BNF, annual grain legumes are second only to cereals (Poaceae) in economic importance as food crops, and perennial herbaceous legumes are some of the most nutritious forages for livestock [1]. Despite all their benefits, less than 15 species of

grain legumes and 50 forage legumes are globally traded and commercially important. This suggests that thousands of species may have been overlooked for their potential utility to humans and unique adaptations to their native environments [7,12].

Domestication and development of new or alternative legume crops could increase crop diversity and reduce human reliance on only a few major food crops, and if done thoughtfully, could improve the resilience and sustainability of food production [13]. Replacing annual with perennial grain crops has been proposed as a solution to improve food and ecosystem security [14]. In contrast to annuals, the deep, extensive root systems and longer growing season of perennials allows them to have increased capacity to capture sunlight and sequester carbon, to reduce moisture and nutrient loss through leaching and/or runoff, and to prevent soil erosion [15–17]. Therefore, new perennial grain legumes, with novel eco-physiological attributes and nutritional properties (i.e., high oil, high protein, high fiber content) similar to those of their annual counterparts, would be valuable additions to the handful of grain legume crops used in modern, sustainable agriculture.

The benefit of including legumes in cropping systems depends on effective nodulation by rhizobia, total BNF and N use efficiency (NUE) [18]. Perennials may have distinct advantages over annuals in this capacity. In annual legume systems, the rhizobia symbiosis must be reestablished every growing season; therefore, the symbiosis only exists for a portion of the plant's lifecycle. As a result, the symbiosis may not wholly supply the annual grain legume's inorganic N requirement and often does little to improve soil N or nutrient status because nearly all BNF N and plant resources are mobilized and translocated to the seed [19]. Conversely for perennial grain legumes, the symbiosis exists and functions during the entirety of each growing season. As a result, perennial legumes benefit from the rhizobia-symbiosis for a much greater proportion of their lifespan; and therefore, are expected to have greater annual rates of BNF and to supply a larger fraction of their inorganic N requirements without further depleting soil N levels. Perennial grain legume production is also likely to have a better NUE than using annual legumes grown as cover crops to supply N to cereal grains. Perennial grain legumes retain the natural synchronicity of N supply and demand during grain fill and have small rates of N loss in the cropping system [17]. In annual cereal grain systems with an annual legume cover crop, the legume may fail to meet the cereal grain's N requirements because the rhizobia-symbiosis exists for only a fraction of the growing season, because using tillage to terminate the legume cover crop can change the carbon-nitrogen balance, because loss of N from the soil occurs due to its volatility and mobility and because complete synchronicity of the legume N supply and the cereal grain N demand is extremely difficult to achieve for maximum productivity [20–22].

Domestication of other non-legume perennial grains is already underway for perennial rice (*Oryza* spp.) [23], perennial wheat (*Triticum* spp.) [24], Kernza[®] (*Thinopyrum intermedium*) [15], Sorghum (*Sorghum* spp.) [25] and *Silphium integrifolium* crops [26]. Some tropical perennial grain legumes already exist and are being grown either commercially or in subsistence settings, such as pigeon pea (*Cajanus cajan*) [27]. Less research has been accomplished and is actively ongoing in breeding and developing a perennial grain legume adapted to temperate climates except for some work involving Illinois bundleflower in the US [28,29] and a screen of potential candidate perennial grain legumes for Australian cropping systems [30,31].

Past efforts to breed and domesticate other perennial grains have generated hypotheses about why annual grains were historically domesticated instead of perennial grains [32] and provided evidence suggesting how current knowledge about the ecology of perennial plants and ecosystems, combined with modern breeding approaches, makes domestication of perennial grains now possible [16,26,33]. Researchers from The Land Institute (Salina, KS, USA) and elsewhere have outlined a pipeline strategy as a guide for grain crop domestication which is composed of three phases (Phase I: Evaluating candidate species; Phase II: Wild species to new crop; and Phase III: From new crop to commodity crop) [34]. Earlier approaches propose candidate screening and selection by determining mean values for desirable traits from a single study or via species-centric approaches that attempt to identify purpose for a promising plant. Instead, the pipeline domestication model attempts to monitor multiple

species' abilities to meet a predefined purpose through multiple phases of selection designed to overcome the limitations that exist for each species [34]. Phase I: Evaluating candidate species, closely resembles a screen for desirable traits or attributes that fit a predefined agricultural target, but more importantly, Phase I aims to identify the primary limitations of each species and to develop specific breeding strategies to address those limitations in Phase II.

Perennial grain legumes are entering Phase I of the pipeline, and the remainder of this review aims to use the ideas developed in the pipeline strategy to outline legume-specific morphological traits or ecophysiological attributes that we assume are desirable for an herbaceous, perennial, temperate-adapted grain legume that is mechanically harvested on a commercial scale. In doing so, we provide relevant data collected for a small group of perennial herbaceous legume species related to the described attributes and suggest a few strategies for evaluating and selecting candidate species to move forward to Phase II of the pipeline.

2. Desirable Morphological and Ecophysiological Attributes of a Temperate-Adapted, Perennial Grain Legume

Plant breeders in established crops have very specific selection criteria and traits that they prioritize in their breeding programs. These criteria are informed by current social, economic, or environmental challenges the crop or industry faces. In plant domestication, the domesticator may not have the luxury of beginning candidate selection or pre-breeding with a complete understanding of all the problems that need to be addressed or even the traits that must be improved. Instead, during Phase I, the most limiting characteristics of each candidate species are identified so that selection criteria can be developed for future phases of the pipeline. Herein we provide some basic concepts that should be considered by domesticators when evaluating candidate species for any alternative cropping system: agronomic potential, seed or end-use quality, ecosystem services provided or required and ease of breeding (Table 1). We then suggest specific morphological and ecophysiological attributes for a temperate-adapted, perennial grain legume in our targeted cropping system that relate to each of these four concepts. Each species is expected to have a unique combination of desirable and undesirable attributes; therefore, the ranking of the relative importance of each attribute will be different for each species and will change as the species moves through the pipeline and as new challenges are revealed. What is more important is that the domesticator has a holistic view of their targeted crop/cropping system and a vision that guides their decision making but is open to change as unforeseen challenges or opportunities arise.

Table 1. Basic concepts and related components that should be considered when evaluating domestication candidates for alternative cropping systems.

Basic Concept	Related Components
Agronomic Potential	Crop Establishment, Field Management, Harvestability, Yield Potential, Adaptability
Seed Quality	Nutritional Profile, Anti-nutritional Factors, High Value Products
Ecosystem Services	Resource Acquisition and Retention, Pollinator Resources, Dual Use, Minimizing Ecosystem Dis-services
Ease of Breeding	Reproductive Biology, Genome Structure, Available Genetic Resources

2.1. Agronomic Potential

Adoption of any new perennial grain legume crop is dependent on its agronomic potential. While genetic improvements in many of our current crops are easily adopted by farmers as new cultivars or varieties, major agronomic innovations and new management practices are adopted more gradually because they often require a steep learning curve and/or expensive purchases of new equipment that are cautiously measured. Therefore, specific traits are desired that allow potential domesticates to be easily grown and managed on a large-scale using machinery and conventional

practices. Many of these traits are part of the same suite of phenotypic transitions that occurred in domestication from annual wild grasses and legumes to today's cereal grain and grain legume crops [35]. We recognize that much of the world that can and would benefit from perennial grain legumes may not use mechanization (e.g., agroecosystems providing for subsistence farmers); it is therefore important that the target agroecosystem should inform the traits that are being selected in any breeding or domestication strategy.

2.1.1. Crop Establishment

Many wild legumes have strong physical seed dormancy with as many as 90% hard seeds (having a seed coat that is impermeable to water) [36]. Because seeding of wild-type, hard seeded legumes results in poor stand establishment, Ladizinsky [35] suggested that wild legumes were not a reliable food source for pre-agricultural humans and were unlikely to be adopted as crop candidates unless free-germinating seeds had been identified. Therefore, perennial legume species exhibiting free-germination or that can be quickly and easily selected to become free germinating will be strong candidates for later phases of the pipeline. Additionally, seedling establishment of perennial forage legumes is slow compared to annuals possibly because they carry high genetic load or they invest in a substantial root system prior to developing above ground foliage [7,33]; however, selection for perennial legumes with divergent root/shoot ratios has been previously accomplished [37]. We expect that increased seedling vigor and effective establishment can be improved via selection in candidates with poor establishment rates.

2.1.2. Field Management

Many best management practices (BMPs) utilized by farmers revolve around identification of crop phenological stages and ease of response to abiotic or biotic pressures. Therefore, excessive morphological or phenological plasticity and indeterminate growth are undesirable traits for a perennial grain legume candidate because they limit management opportunities. Continuous vegetative and reproductive growth, indeterminacy, is characteristic of wild legumes; and selection for determinacy is recognized as part of the grain legume domestication syndrome [35,38]. Choosing potential domesticates that have synchronous growth, flowering and fruit ripening ensures that farmers can fertilize, pollinate and harvest their crop in fewer visits. Likewise, legumes that have a creeping, prostrate, vining, or rambling growth habit are not ideal candidates because they are burdensome to manage in breeding nurseries and in field production (Table 2). Vining forms of some common grain legumes (e.g., peas, soybeans, common beans) are still grown in subsistence settings or home gardens; however, almost all commercial production has turned to more erect, non-vining forms to avoid trellising and to allow for large-scale field management and harvest. In soybean, determinacy is controlled by two loci, *Dt1* (homolog of *Arabidopsis TERMINAL FLOWER1*) and *Dt2* [39,40]; and loci for non-twining (i.e., bush type) growth have also been identified [41]. Therefore reverse genetic approaches using TILLING (targeting induced local lesions in genomes) or genome editing to target these genes could be a feasible solution to the issue in exceptionally promising domesticates [42].

Table 2. Temperate-adapted perennial grain legume candidates and their respective growth habits and inflorescence type/placement which may affect their agronomic potential.

Species	Growth Habit	Inflorescences	References
<i>Apios americana</i>	Twinning, rhizomes	Axillary racemes	[43]
<i>Astragalus canadensis</i>	Erect, rhizomes	Axillary racemes	[43]
<i>Astragalus cicer</i>	Decumbent to erect, rhizomes	Axillary racemes	[43]
<i>Astragalus crassicaarpus</i>	Decumbent to erect	Axillary racemes	[43]
<i>Baptisia australis</i>	Decumbent to erect, rhizomes	Terminal raceme	[43]
<i>Dalea purpurea</i>	Erect	Terminal spikes	[43]
<i>Desmanthus illinoensis</i>	Decumbent to erect	Axillary heads	[43]
<i>Desmodium canadense</i>	Erect	Panicle of racemes	[43]

Table 2. Cont.

Species	Growth Habit	Inflorescences	References
<i>Desmodium glutinosum</i>	Erect	Terminal or panicle of racemes	[43]
<i>Desmodium illinoense</i>	Erect	Terminal elongated raceme or panicle of raceme	[43]
<i>Desmodium sessilifolium</i>	Erect	Panicle of racemes	[43]
<i>Glycyrrhiza glabra</i>	Erect, rhizomes	Axillary racemes	[44]
<i>Glycyrrhiza lepidota</i>	Erect, rhizomes	Axillary racemes	[43,45]
<i>Lathyrus japonica</i>	Creeping, stoloniferous	Axillary racemes	[46]
<i>Lathyrus tuberosus</i>	Erect, rhizomes with small tubers	Axillary racemes	[46]
<i>Lupinus argenteus</i>	Erect	Terminal racemes	[43]
<i>Lupinus leucophyllus</i>	Erect	Terminal racemes	[47]
<i>Lupinus nootkatensis</i>	Erect, bush	Terminal raceme	[48]
<i>Lupinus perennis</i>	Erect	Terminal racemes	[49]
<i>Lupinus polyphyllus</i>	Erect, rhizomes	Terminal raceme	[50]
<i>Lupinus rivularis</i>	Erect	Terminal raceme	[51]
<i>Lupinus sericeus</i>	Erect	Terminal raceme	[52]
<i>Medicago sativa</i>	Decumbent to erect	Axillary racemes	[43]
<i>Onobrychis transcaucasica</i>	Erect	Terminal raceme	[53]
<i>Onobrychis viciifolia</i>	Erect	Spike raceme	[43]
<i>Oxytropis lambertii</i>	Erect	Terminal raceme	[43]
<i>Pedimelum esculentum</i>	Erect	Terminal raceme	[43]
<i>Pedimelum tenuiflorum</i>	Erect	Axillary racemes	[43]
<i>Phaseolus polystachios</i>	Vining	Axillary racemes	[54]
<i>Senna marilandica</i>	Erect, rhizomes	Terminal and upper axillary racemes	[43]
<i>Thermopsis villosa</i>	Erect	Terminal raceme	[55]
<i>Thermopsis montana</i>	Erect	Terminal raceme	[56]
<i>Trifolium pratense</i>	Decumbent to erect	Terminal heads	[43]
<i>Vicia americana</i>	Sprawling to climbing	Axillary racemes	[43]
<i>Vicia cracca</i>	Trailing to climbing	Axillary racemes	[46]
<i>Vicia nigricans</i>	Sprawling to climbing	Axillary racemes	[57,58]
<i>Vicia pisiformis</i>	Climbing	Axillary racemes	[59]

2.1.3. Harvestability

Non-shattering (loss of the seed dispersal mechanism such as pod dehiscence), is considered one of the crucial grain domestication traits because it prevents excess loss of seed, immediately increasing harvestable yield [60,61]. Pod indehiscence is common in grain legumes, and pod dehiscence should not be a disqualifying characteristic of candidate species because it has been routinely selected against in recent legume domestications [29,62]. Plants with an erect, non-lodging growth habit and large, dense, smooth seeds would be ideal for mechanized harvest [34] (Table 3). The natural indeterminacy and lack of synchronous leaf senescence during seed maturation causes many perennial grain legumes to “stay green” throughout the entire growing season. The moisture in the green plant tissues introduces problems for mechanical harvest. Ideally, selection candidates that undergo a programmed senescence corresponding to seed maturation could be identified or easily selected; however, that may be unlikely and other strategies should be identified. Seed production of many perennial forage legumes requires precutting or plant desiccants to aid in “drying down” the plant prior to mechanical harvest [63,64], but the extra field pass and the use of chemical inputs should be avoided if possible. Candidate species with terminal racemes which extend above the leaf canopy, as opposed to axillary racemes, could allow for mechanical harvest by avoiding contact between the cutting machinery and the higher moisture plant material lower in the canopy.

Table 3. Temperate-adapted, perennial grain legume candidates and traits that may affect their harvestability and yield potential. These data are from multiple studies and the phenotypes could vary greatly across environments.

Species	Fruit Length (mm)	Seed Length (mm)	1000 Seed Weight (g)	Seeds per Pod	References
<i>Apios americana</i>	40–100	4.0–5.0		4–6	[43,58]
<i>Astragalus canadensis</i>	10–20	1.5–2.5	2.0		[43,47,58,65,66]
<i>Astragalus cicer</i>	10–15	2.4–2.6	3.4		[43,66,67]
<i>Astragalus crassicaarpus</i>	15–40	2.0–4.0	6.6		[43,66]
<i>Baptisia australis</i>	20–60	3.5–5.0	16.1	1–2	[43,58,66]
<i>Dalea purpurea</i>	2–2.5	1.5–2.0	3.2	1	[43,65,66]
<i>Desmanthus illinoensis</i>	10–25	3.0–5.0	6.0	6	[43,65,66]
<i>Desmodium canadense</i>	5–7	3.5–5.0	5.1	1–5	[43,58,66]
<i>Desmodium glutinosum</i>	24–36	6.0–7.0	17.3	1–3	[43,65,66]
<i>Desmodium illinoense</i>	32–56	3.0–3.5	6.6	3–7	[43,65,66]
<i>Desmodium sessilifolium</i>	12–20	2.5–3.5	3.7	1–3	[43,58,65,66]
<i>Glycyrrhiza glabra</i>	10–30		6.1	2–8	[46,66,68]
<i>Glycyrrhiza lepidota</i>	10–20	2.5–4.0	7.0	3–5	[43,65,66]
<i>Lathyrus japonicus</i>	40–60	4.0–4.5	27.9	5–8	[46,66,69]
<i>Lathyrus tuberosus</i>	20–40		30.9		[46,66]
<i>Lupinus argenteus</i>	10–30	3.7–4.5	27.1	4–6	[43,65,66]
<i>Lupinus leucophyllus</i>	15–30	4.2–5.6	9.9	3–6	[47,66,70]
<i>Lupinus nootkatensis</i>	50–60	3.5–4.2	10.6	10–11	[66,68,70]
<i>Lupinus perennis</i>	30–50		22.1	5–6	[49,66,70]
<i>Lupinus polyphyllus</i>	25–40	6.0	21.0	3–9	[50,66,68]
<i>Lupinus rivularis</i>	50		36.0	6–10	[51,71]
<i>Lupinus sericeus</i>	20–30	4.0	22.3	2–5	[52,65,66]
<i>Medicago sativa</i>	4–8 (coil diameter)	2.0–3.0	2.0	2–12	[43,66,68]
<i>Onobrychis transcaucasica</i>	6	3.0–3.2	10.2	1	[66,72]
<i>Onobrychis viciifolia</i>	5–8	4.0–7.0	18.3	1	[43,66]
<i>Oxytropis lambertii</i>	5–6	2.0	1.6		[43,66]
<i>Pedimelum esculentum</i>	20	4.0–6.0	20.7	1–2	[43,66]
<i>Pedimelum tenuiflorum</i>	5–9	4.0–5.5	35.6		[43,66]
<i>Phaseolus polystachios</i>	30–60	6.0–10.0	60.9	4–6	[58,66,68,73]
<i>Senna marilandica</i>	65–100	4.5–5.5	19.6	10–25	[43,66]
<i>Thermopsis villosa</i>	40–55	3.0–3.5		7–12	[55,66]
<i>Thermopsis montana</i>	45–65	3.5–5.0	18.1	6–16	[56]
<i>Trifolium pratense</i>	3	1.5–2.0	1.3	1	[43]
<i>Vicia americana</i>	25–40	4.0–5.0	16.8	2–12	[43]
<i>Vicia cracca</i>	20–25	2.5–2.9	13.8	3–6	[46,66,74]
<i>Vicia nigricans</i>	20–45		34.6–89.9		[57,66]
<i>Vicia pisiformis</i>		4.5–4.7	41.6		[46,66,74]

2.1.4. Yield Potential

Yield potential (the maximum attainable yield for a specific variety in an environment where it is preadapted) is driven by total biomass production and harvest index (ratio of grain mass to total above ground biomass) [34,75]. By harvesting a much larger portion of the seasonally available water, nutrients and photosynthetic energy, many perennials achieve higher net primary productivity in contrast to annuals in both natural and agro-ecosystems [24,76]. Therefore, promising perennial grain legume candidates will be highly productive during the entirety of the season and capable of producing large quantities of biomass while persisting in the cropping system for multiple years [77,78]. Sun-adapted legumes may be more productive than shade-adapted legumes in agricultural settings by becoming light saturated at higher levels of photosynthetic active radiation (having a greater radiation use efficiency), thus having higher maximal carbon assimilation rates [79].

Wild perennials generally produce lesser quantities of seed of smaller size compared to annuals via lower harvest indices (Table 3). The later successional habitats of perennial species favor plants that allocate resources to heterotrophic tissues or increases in total size. Thus, perennials tend to be more competitive and longer-lived but at the expense of reproductive output [80]. By moving perennials to more favorable agricultural environments and applying selection pressure for increased seed yield,

we expect to elevate the yield potential of perennial domesticates by increasing their harvest index as has been done in annual grains and more recently in the perennial grain, Kernza[®] [81]. Even so, perennials with high overall fecundity are especially desirable candidates. Lastly, while many wild herbaceous perennial legumes and cultivated forage legumes are entomophilous and allogamous [7], the preferred domesticates would be capable of self-pollination and autogamy to ensure high yields by reducing dependence on pollinators.

2.1.5. Adaptability

Alfalfa is a great example of a widely adapted perennial candidate; its broad adaptation to a wide range of soils and climates has made it the dominant perennial forage legume and expanded its native range from Caucasia and Central Asia to all continents except Antarctica [82]. Opportunities exist to develop alternative forage and grain legumes adapted to specific harsh environments, for example legumes adapted to acidic and infertile soils are being screened in Australia [83]. However, because developing even a single, temperate-adapted perennial grain legume will require substantial investment, broadly adapted (multiple soils, climates and geographies) or broadly adaptable domesticates that are productive with minimal input requirements are preferred [34]. Similarly, legumes that have escaped cultivation and/or become naturalized outside of their native range suggest a certain degree of adaptability (Table 4). Broad adaptation is more complicated in perennials than annuals in temperate climates because the perennial species must survive multiple years through seasons of changing temperatures, photoperiods and precipitation patterns. In contrast, annual plants must survive only through the few months of suitable conditions [7].

Table 4. Temperate-adapted, perennial grain legume candidates, their native or naturalized range and their preferred soil/habitat.

Species	Native or Naturalized Range	Native Soil/Habitat	References
<i>Apios americana</i>	Great Plains	moist prairie ravines, pond and stream banks, thickets	[43]
<i>Astragalus canadensis</i>	Great Plains	moist prairies, woodlands, roadsides, thickets, stream banks	[43]
<i>Astragalus cicer</i>	Europe; cultivated	slightly acidic to moderately alkaline	[43]
<i>Astragalus crassicaarpus</i>	Great Plains	rocky/sandy prairie hillsides/uplands	[43]
<i>Baptisia australis</i>	Great Plains	rocky/sandy prairie, rocky open woods, limestone glades, stream valleys	[43]
<i>Dalea purpurea</i>	Great Plains	dry prairie	[43]
<i>Desmanthus illinoensis</i>	Great Plains	dry to moist prairie, wooded slopes, wasteland	[43]
<i>Desmodium canadense</i>	Great Plains	sandy soil	[43]
<i>Desmodium glutinosum</i>	Eastern Great Plains	woodlands	[43]
<i>Desmodium illinoense</i>	Central Great Plains	rich prairie soils	[43]
<i>Desmodium sessilifolium</i>	Southeast Great Plains	dry or sterile woodlands, hillsides, ravines, valleys	[43]
<i>Glycyrrhiza glabra</i>	Eurasia; cultivated and naturalized in US	cultivation, ruderal sites	[68]
<i>Glycyrrhiza lepidota</i>	Great Plains	moist, fertile prairie, shores, meadows, wasteland	[43]
<i>Lathyrus japonicus</i>	Circumpolar, North America, South America, Eurasia	coastal shores, beaches	[46,69]
<i>Lathyrus tuberosus</i>	Temperate Eurasia; introduced in Northeast North America	moist meadows, riparian	[46]
<i>Lupinus argenteus</i>	Northwestern Great Plains	prairies, roadsides, open woodlands	[43]
<i>Lupinus leucophyllus</i>	Western North America	open forests, grasslands, sagebrush, roadsides	[68]
<i>Lupinus nootkatensis</i>	Western Canada, Alaska (introduced mainland US, Iceland)	gravel bars, meadows, tidal marshes, open slopes, cultivated, escaped	[47,68]

Table 4. Cont.

Species	Native or Naturalized Range	Native Soil/Habitat	References
<i>Lupinus perennis</i>	Eastern US		[49]
<i>Lupinus polyphyllus</i>	Western North America	moist soils	[50]
<i>Lupinus rivularis</i>	Western North America	well-drained, sandy soils	[51]
<i>Lupinus sericeus</i>	Western North America	grasslands, forests	[52]
<i>Medicago sativa</i>	Europe & Western Asia; cultivated and naturalized world wide	all soils, neutral pH	[43]
<i>Onobrychis transcaucasica</i>	Caucasus; cultivated	dry slopes	[53]
<i>Onobrychis viciifolia</i>	Europe & Western Asia; cultivated	dry, calcareous soils	[43]
<i>Oxytropis lambertii</i>	Great Plains	dry, upland prairie	[43]
<i>Pedimelum esculentum</i>	Great Plains	dry soils	[43]
<i>Pedimelum tenuiflorum</i>	Great Plains	dry prairie	[43]
<i>Phaseolus polystachios</i>	Eastern and Southcentral US	moist woodlands, near streams, roadsides, upland woodlands, clearings	[68]
<i>Senna marilandica</i>	Southeast Great Plains	sandy, moist soils	[43]
<i>Thermopsis villosa</i>	East TN, West NC	woodlands	[55]
<i>Thermopsis montana</i>	Western US	moist meadows	[56]
<i>Trifolium pratense</i>	Southern Europe; cultivated and naturalized in US	heavy, fertile, well-drained soils	[43]
<i>Vicia americana</i>	Great Plains	uplands, badlands, bluffs, wasteland	[43]
<i>Vicia cracca</i>	Eurasia; introduced widely in North America	forest edge, scrubland, lowland, grassland, slopes, moist sites	[46]
<i>Vicia nigricans</i>	Western North America	coastal forest and shrubland, chaparral	[57]
<i>Vicia pisiformis</i>	Central and East Europe; introduced elsewhere	forested steppes	[59]

Information about a candidate species' population ecology may provide evidence of future unforeseen limitations, such as the potential for insect and disease pressure. In native grasslands, many legume and forage species occur as isolated plants or small clumps of plants spread across the landscape [84], possibly allowing the plants to escape insect predation or disease pressure by simply lacking apparency (visibility to potential herbivores) [85,86]. However, when planted at higher densities necessary for production agriculture, a candidate legume's apparency will be increased and could allow for major insect infestations or disease epidemics on farms and in breeding nurseries [26,87]. Species that are more apparent or are preadapted to living at higher population densities might thus be promising candidates. Species that experience substantial disease pressure or insect predation in their native ranges regardless of their population densities or apparency, such as *Baptisia leucophaea* and *B. leucantha*, should be avoided [88].

To realize the advantages of a perennial grain legume over other perennial or annual grain crops, it is essential that maximum BNF and productivity occur under low input conditions. Managing the legume-rhizobia symbioses is of primary importance, and selection and domestication of candidate legume species and the appropriate rhizobia strain must occur in parallel to ensure the adaptation of both to the target environment and cropping system. The process of isolating new rhizobia strains, testing their effectiveness on a broad range of hosts, releasing the effective strains, and monitoring their success across a broad range of climates and soil types is no small task and should not be overlooked [7]. Candidates that nodulate and achieve optimal BNF when inoculated with already commercially available rhizobia strains may advance more quickly through the domestication pipeline, but candidates should not be dropped from the pipeline simply because strain isolation and selection is required.

2.2. Seed Quality

Grain legumes, in their domesticated forms, have been major components of human diets for thousands of years, and the majority of the grain legumes grown today are the same ones that were known and grown by disparate ancient civilizations. In recognition of the historical importance of grain legumes (pulses) and in anticipation of their future role in ensuring food and nutritional security and maintaining soil fertility, the United Nations Food and Agriculture Organization (FAO) declared 2016 the International Year of the Pulses, with a subtitle of Nutritious Seeds for the Future. In line with the FAO's vision for pulses, our interest in developing new perennial grain legumes is likewise focused on finding potential domesticates that contribute to the health of both the soils where they are grown and the people that they feed. Understanding seed chemistry, composition and utility is required to assess potential candidate species and to identify any food quality concerns or opportunities that should be targeted in future breeding efforts.

2.2.1. Nutritional Profile

Seeds of grain legumes are highly nutritious and have high protein content (ranging from 17 to 30 percent dry weight), with seeds of some species and varieties containing nearly twice as much protein as cereal grains [89]. A major global source of plant-based proteins, they provide ten percent of the dietary requirements of proteins worldwide [90]. As such, grain legumes are significant components in the diets of people living within subsistence farming communities, in parts of the world where animal proteins are scarce or expensive and of those who choose to be vegetarians [91]. Pulses are also important sources of energy in the form of oil and carbohydrates. Legume carbohydrates include substantial quantities of starch, oligosaccharides and an especially high dietary fiber content [91]. Oil content is variable among grain legume seeds ranging from one percent oil content in some species to more than 30% in species like soybean, peanuts and lupins [92]. We expect that the seeds of most wild legumes will be nutritious, and the nutritional profile of potential domesticates should be at least partially considered as a criterion for evaluation with preference given to species whose seeds are high in protein and oil.

2.2.2. Anti-Nutritional Factors

A major constraint to developing nutritious perennial grain legumes for human consumption is the prevalence of anti-nutritional factors in seeds of wild legumes such as non-protein amino acids (e.g., canavanine [93]), quinolizidine alkaloids [94], glycosides, tannins, saponins, and protease inhibitors [95]. We expect almost every candidate species, including those with ethnobotanical evidence of previous human consumption, to contain one or more compounds that should be removed via breeding or post-processing to maximize protein digestibility and to ensure edibility. Seeds of many annual grain legumes have traditionally been processed via soaking, leaching, boiling, or fermenting to remove the anti-nutritional compounds; and some of these legumes, like common bean, still need to be soaked and cooked to ensure normal digestion and metabolism [95]. Some anti-nutritional factors are under the control of one or a few large-effect genes, as is the case for alkaloid biosynthesis in lupins [96]. While this suggests that selection may be effective for eliminating some compounds in some species, combining all the required genes for domestication is not trivial. Furthermore, many legume anti-nutritional compounds act as deterrents against insect pests or act as important N storage compounds in seeds; therefore, breeding for a reduction in those compounds may make the plants more susceptible to herbivory or less nutritious for human consumption [96,97]. Each potential domesticate is likely to present a unique scenario and require a unique decision as to whether to pursue a breeding, processing, or combined strategy to ensure palatability.

2.2.3. High Value Products

Because of their nutrient profile (high protein, oil and fiber content), seeds of grain legumes present tremendous opportunity to be used in a variety of processed human food products or as livestock or aquaculture feed [7,90]. If the domesticate has a similar nutritional profile and the functional attributes of an existing annual grain legume, immediate market opportunities could be available for its commercialization as a substitute without an extensive marketing strategy. However, if seed of the domesticate has a unique flavor, contains unique phytochemicals and can be processed into some high-value or specialty product, development of the crop may be easier from a funding and marketing standpoint [34]. Some of the anti-nutritional factors in wild domesticates may function as these high-value products as evidenced by recent efforts to utilize alfalfa and soybeans as sources of phytoestrogens and to identify novel antioxidant and antimicrobial compounds in native legume species [98–101]. Finally, we expect that the novel perenniality of any potential domesticate will add value to the crop because of the various ecosystem services this life form provides [76].

2.3. Ecosystem Services

Crop yields were likely the primary concern in the domestication of the suite of annual grains available for modern agriculture. In many cases, we continue to willingly increase water, fertilizer and fossil fuel use to maximize their productivity in our cropping systems. With the domestication of perennial grains, we have the opportunity to develop a new suite of crops that maximize both the yields and the sustainability of the cropping systems in which they are deployed. The development of new perennial grains is driven by the need for renewable, sustainable agricultural commodities whose production limits negative anthropogenic effects on the environment and provides positive ecological benefits. Measurable benefits to humans include soil conservation, landscape restoration, restoration of nutrient and hydrological cycles and increased biodiversity [102]. Therefore, the potential ecosystem services provided by any domesticate should be monitored closely, enhanced through breeding and promoted as beneficial and necessary attributes.

2.3.1. Resource Acquisition and Retention

N is the most critical limiting element for plant growth, and availability of sufficient N is essential for producing high-quality, protein rich, plant-based foods [103]. Synthetic N fertilizers are common expensive inputs costing agriculture more than \$45 billion US each year. Synthesis of those N fertilizers through the Haber-Bosch process represents 1–2% of the world's total energy consumption and directly releases more than 300 Tg of fossil fuel derived CO₂ into the atmosphere annually [21,104,105]. Furthermore, the mobility of the applied inorganic N fertilizers results in less than 50% fertilizer N-recovery efficiency by the first crop with substantial amounts of the remaining N leaving the cropping system as N₂O and NO₃ which have environmental impacts elsewhere [105,106].

Perennial grain legumes would be uniquely positioned as crop plants which are able to sustainably produce high protein foods by having specialized strategies for acquiring and retaining N within the cropping system for themselves and for subsequent crops within the rotation. Specifically, N acquisition through the plant-rhizobia symbiosis results in N that is directly incorporated into the growing plant, overcoming problems of low fertilizer N-recovery efficiency in other annual grain cropping systems. Furthermore, while N₂ fixation in legumes is considered to have higher energy and carbon (C) requirements than N assimilation by plants using reduction of NO₃ for growth, the energy is supplied via solar radiation rather than through fossil fuels; thus the resulting CO₂ respired by the nodules originates through photosynthesis and is not a net contributor to atmospheric CO₂ concentrations [104, 106]. Once acquired, fixed N is likely to be better retained in a perennial grain legume cropping system for two reasons. First, N deposited into the soil via plant residues occurs in immobile, organic forms with longer mean residence times than synthetic N additions [106,107]. Secondly, perennial grain legumes have an additional retention strategy in that, as the organic N is mineralized by microbes, the

N can be reassimilated by the plants via their large, perennial root systems that actively take up N during a prolonged growing season [24,104,107]. Finally, perennial legumes are especially useful in acquiring and retaining N for use by later rotational crop species. For example, N credits following alfalfa are estimated to range up to 170 kg per ha [108].

In addition to BNF, the members of the legume family display a range of adaptations for the acquisition and retention of other important resources. Preferred domesticates should have excellent resource use efficiency and be productive even in low input environments. Many perennial species, by virtue of their large active root systems and specialized root structures (tubers or crowns), are able to efficiently capture, respond to and/or store available water [15,109]. Some potential alternative legumes are already being evaluated specifically for that ability [78,83]. Additionally, other legumes have strategies for acquiring phosphorous (the second most limiting element to plant growth behind N) via specialized root structures (e.g., cluster roots), by releasing carboxylates into surrounding soils and through arbuscular mycorrhizal associations [110,111]. Lastly, the large rooting systems of perennial legumes function to increase soil organic C by reducing erosion, reducing microbial respiration (via lack of tillage) and by adding large amounts of C back into the system. As a result, nutrients are retained in the cropping system for use by the current and subsequent crops [20,112]. Preferred legume candidates will have one or more of these nutrient acquisition and/or retention strategies, will maintain and build the soil fertility in the field where it is grown, and thus, will improve the productivity for the subsequent rotational species.

2.3.2. Pollinator Resources

Crop pollination, via animals and especially insects, is regarded as one of the key services that natural ecosystems provide for humans and that is essential to human welfare [113]. Bees in particular are estimated to be essential for as much as 30% of the world's food production, which relies on wild and managed pollinators for successful fruit and seed set [114,115]. Bees are necessary for the reproductive success of many of the herbaceous perennial forage legumes that are allogamous and entomophilous [7], and bee pollination will be required for high perennial grain legume yields unless autogamous candidates are identified or developed via breeding. Agricultural intensification and modern land use patterns are disrupting pollinator communities and leading to a decline of native bee populations in many areas of the world [116]. Dependent on bees themselves, the perennial grain legumes might also provide floral resources necessary for maintaining and rebuilding native bee populations in agricultural areas so that neighboring farms and natural areas might also benefit from enhanced pollination services [116]. Domesticates with elevated nectar and pollen production, specific flower colors, or certain floral morphologies that attract a wide variety of pollinators may be useful.

2.3.3. Dual-Purpose Legumes

Legume candidates that produce large quantities of harvestable biomass, in addition to grain, might be adopted as crops sooner than those that produce grain alone by having improved economic viability and versatility for farmers. In many parts of the world, annual grain legumes are utilized as dual-purpose crops producing grain for human consumption and crop residues as feedstocks for livestock or bioenergy [117]. Grain legumes with highly palatable and nutritious leaves and stems residues could fit within similar crop/livestock systems if their crop residues are free of anti-nutritional factors [118].

2.3.4. Minimizing Ecosystem Dis-Services

Due diligence is required to determine the broader biological implications and threats of domesticating new perennial grain legumes and releasing both the plants and their rhizobia microsymbionts into areas within and outside their centers of origin. Crop candidates should be evaluated for their likelihood to introduce ecosystem dis-services such as competition for water, pollination, or other resources from local ecosystems [119]. Likewise, species that require large

quantities of pesticides to manage insect, disease, or weed issues should be avoided to prevent harm to non-target species and to prevent pest problems for other crops in the same growing region [120].

Newly domesticated legume species capable of higher rates of N₂ fixation and with increased fitness owing to cycles of artificial selection, intraspecific hybridization, or reduction in genetic load may be predisposed to becoming invasive outside their native ranges without their native biotic controls [34,121]. Species which have already become invasive or weedy outside the native range should be considered cautiously. Because the potential invasiveness and the ecological ramifications of invasion by each candidate are unknown and difficult to predict, special precautions should be made during the candidate evaluations and later phases of the domestication pipeline to ensure that genetic pollution and/or introduction of foreign germplasm does not occur [34].

2.4. Ease of Breeding

No domesticate is expected to have all, or even the majority, of the morphological or ecophysiological attributes necessary for a temperate-adapted perennial grain legume. Artificial selection will be required to identify genetic variation for domestication traits and combine them into single populations and/or genotypes. A species' ease of breeding will be likely to determine how long it takes to pass through the domestication pipeline and to be released as a new crop. Factors that influence breeding ease include the species' reproductive biology, genome structure, and availability of genetic resources; and these should be considered in the candidate evaluation process [34].

2.4.1. Reproductive Biology

Compared to other crops, remarkably little is known about the reproductive biology of cultivated perennial forage legumes and even less is known about reproduction within wild, herbaceous perennial species [7]. Many perennial legumes are allogamous, meaning they must be cross-pollinated to produce seeds either because they have self-incompatibility systems [122,123], have accumulated genetic load [124], exhibit dichogamy, or require mechanical tripping. The higher levels of genetic diversity maintained in cultivars of allogamous (outcrossing) compared to autogamous (self-pollinated) species may give them certain ecosystem service benefits like disease and pest control [125]. However, the same features that maintain genetic diversity in allogamous candidates will cause fixation of domestication syndrome traits (and movement through the pipeline) to proceed much more slowly than in autogamous taxa [126]. Furthermore, breeders (and eventually seed producers) of allogamous crops must undertake elaborate measures at each seed increase stage to produce pure seed in isolation using controlled pollination by insects or another appropriate vector; conversely, it is much easier to produce large quantities of genetically pure seed for autogamous species [7].

The vast size of the legume family and its variety of floral adaptations is often attributed to its coevolution with pollinating bees [127], whose ability to recognize numerous complex shapes and colors may have resulted in the evolution of unique floral morphologies that attract specific pollinators [128]. The floral adaptations of some legume species may affect our ability to perform controlled crosses between pairs of plants and thus decrease the ease of breeding for that candidate. Members of the Caesalpinioideae subfamily are variable in floral morphology while those from the mimosoid clade tend to be actinomorphic; however, most Papilionoideae species have bilaterally symmetrical (zygomorphic) flowers with the pistil and stamen hidden within abaxial (keel) petals [129]. Species with very small flowers from any of the three clades or whose reproductive organs are difficult to access (especially in the Papilionoideae) may require elaborate time-consuming techniques for emasculation or necessitate the use of genetic tools to identify F₁ individuals. Allogamous species with functioning self-incompatibility systems may allow for paired pollination of plants without emasculation, but autogamous species with large, easy to emasculate flowers will be preferred. Furthermore, a large variation in mean ovule number (e.g., *Onobrychis viciifolia* = 1.0 ± 0.0, *Lotus corniculatus* = 56.3 ± 5.5) and mean pollen grain number (e.g., *Anthyllis vulneria* = 3654 ± 948, *Lotus corniculatus* = 198,500 ± 13,012) per flower exists between perennial legume species [130]. Candidate

species that produce sufficient amounts of pollen and that have many ovules per flower are preferred so that each hand pollination or controlled cross produces abundant seeds.

The domestication timeline for most perennial grain candidates will be limited by the annual rates of genetic gain that are achieved. Plants with prolonged juvenile periods prior to reproductive maturity will require breeding cycles that span multiple years and should be avoided. Perennial species, especially temperate-adapted perennials, have floral induction pathways that are modified via photoperiod or chilling temperatures to ensure that they flower during the appropriate season [131]. In most cases, these traits are necessary to ensure the long-term success of perennial grains as crop plants. However, domesticates that can be grown in the greenhouse, that are easily induced to flower under artificial lighting and/or temperatures, or that flower under normal greenhouse conditions will allow for comparatively more cycles of selection per unit of time than those that cannot [34].

2.4.2. Genome Structure

Advances in DNA sequencing technologies have made the development of genetic and genomic resources and their application in molecular-assisted breeding strategies possible and affordable, even in alternative non-model crops. Molecular breeding and statistical genomic approaches, particularly genomic prediction, could be useful tools for accelerating the domestication process for new crops, particularly for perennial species with multi-year breeding cycles or that require multiple years of phenotyping prior to selection [26,132]. Genomic-assisted breeding approaches will likely be cheaper and easier to apply to candidate species with small, noncomplex genomes (Table 5). Addressing biological questions using sequencing approaches in domesticates with large, complex genomes will require greater coverage, larger minimum read depths and more computationally demanding analyses [133,134].

Ploidy of potential domesticates should also be taken into consideration because sequence assembly and genotyping is much cheaper and easier in diploids than at higher ploidy levels [135]. While some molecular approaches involving allopolyploids with disomic inheritance may be similar in complexity to those performed in diploids, autopolyploids require complex approaches for marker/sequence polymorphism detection and dosage estimation [135,136]. Finally, classical breeding approaches in autopolyploids require many more plants to be grown to identify unique segregates. For example, consider a particular domestication trait under the control of just two genes, both of which must be in the complete recessive state. Expected segregation ratios in the F_2 generation suggest that in an autotetraploid with tetrasomic inheritance, at least 1296 progeny need to be grown to identify a single plant that has the recessive allele for both genes in all four chromosomes. Conversely, a diploid or allotetraploid with disomic inheritance would require just 16 progeny to be grown [137]. As such, preferred candidates will have small, diploid genomes (Table 5).

Table 5. Temperate-adapted, perennial grain legume candidates, their ploidy level(s) and the number of accessions available through the United States Department of Agriculture National Plant Germplasm System (USDA NPGS) [138].

Species	Ploidy	Accessions in the USDA NPGS	References
<i>Apios americana</i>	$2n = 2x = 22$ & $2n = 3x = 33$	0	[139]
<i>Astragalus canadensis</i>	$2n = 2x = 16$	14	[43,140]
<i>Astragalus cicer</i>	$2n = 2x = 32$ & $2n = 4x = 64$	116	[141]
<i>Astragalus crassicaarpus</i>	$2n = 2x = 22$	3	[43,140]
<i>Baptisia australis</i>	$2n = 2x = 18$	5	[43]
<i>Dalea purpurea</i>	$2n = 2x = 14$	10	[43,140]
<i>Desmanthus illinoensis</i>	$2n = 2x = 28$	50	[43]
<i>Desmodium canadense</i>	$2n = 2x = 22$	4	[43,140]
<i>Desmodium glutinosum</i>	$2n = 22$	0	[43]

Table 5. Cont.

Species	Ploidy	Accessions in the USDA NPGS	References
<i>Desmodium illinoense</i>	2n = 22	0	[43]
<i>Desmodium sessilifolium</i>	2n = 22	1	[43,142]
<i>Glycyrrhiza glabra</i>	2n = 2x = 16	3	[143]
<i>Glycyrrhiza lepidota</i>	2n = 2x = 16	7	[43,140]
<i>Lathyrus japonicus</i>	2n = 2x = 14	7	[144]
<i>Lathyrus tuberosus</i>	2n = 2x = 14	10	[144]
<i>Lupinus argenteus</i>	2n = 2x = 48	25	[43,140,145]
<i>Lupinus leucophyllus</i>	2n = 2x = 48 & 2n = 4x = 96	39	[145]
<i>Lupinus nootkatensis</i>	2n = 2x = 48	0	[146]
<i>Lupinus perennis</i>	2n = 2x = 48	1	[147]
<i>Lupinus polyphyllus</i>	2n = 2x = 48	20	[148,149]
<i>Lupinus rivularis</i>	2n = 2x = 48	5	[150]
<i>Lupinus sericeus</i>	2n = 2x = 48	19	[149]
<i>Medicago sativa</i>	2n = 4x = 32	3529	[43,151]
<i>Onobrychis transcaucasica</i>	2n = 2x = 14	134	[72]
<i>Onobrychis viciifolia</i>	2n = 4x = 28	161	[72]
<i>Oxytropis lambertii</i>	2n = 48	8	[43]
<i>Pedimelum esculentum</i>	2n = 2x = 22	0	[43,140]
<i>Pedimelum tenuiflorum</i>	2n = 22	0	[43]
<i>Phaseolous polystachios</i>	2n = 2x = 22	2	[152]
<i>Senna marilandica</i>	2n = 28	2	[43,153]
<i>Thermopsis villosa</i>	2n = 2x = 18	0	[55]
<i>Thermopsis montana</i>	2n = 2x = 18	5	[55]
<i>Trifolium pratense</i>	2n = 2x = 14 & 2n = 4x = 28	1066	[43]
<i>Vicia americana</i>	2n = 2x = 14	1	[43,154]
<i>Vicia cracca</i>	2n = 2x = 14 & 2n = 4x = 28	4	[155]
<i>Vicia nigricans</i>	2n = 2x = 14	0	[144]
<i>Vicia pisiformis</i>	2n = 2x = 12	1	[155]

2.4.3. Available Genetic Resources

Plant genetic resources are critical sources of genetic variation necessary for increasing nutritive value, yield potential and resilience of crop species through artificial selection. Successful domestication, breeding and adaptation of species as crop plants has often been attributed to the availability and maintenance of genetic diversity within the species, with maize being perhaps the best example [156]. Therefore, beginning a plant domestication program with numerous germplasm resources representing broad levels of diversity within the target species' gene pool is both desirable and perhaps necessary to identify genetic variation for domestication traits [13,26]. Unfortunately, wild perennial species tend to be poorly collected and represented in germplasm collections [157], and germplasm for initial evaluations of some candidate species will likely need to be obtained through native plant nurseries or to be collected directly from wild populations (Table 5). Because they often have substantially more genetic resources available than wild candidates, perennial forage legumes might be especially promising candidates for early Phase I evaluations (Table 5). Furthermore, previous breeding, agronomic, or genetic research may already have been completed to overcome some of the potential limitations (Phase II goals) for the forage legume species.

In addition to the availability of intraspecific genetic variation, interspecific genetic variation that is available for a candidate during its domestication may also prove useful in further expanding its gene pool. This will be especially true if the candidate is a crop wild relative or is in the secondary gene pool of an already domesticated grain crop. These candidates could benefit via direct introgression of domestication traits from their crop relatives. Alternatively, if the relative is reproductively isolated from the candidate but has abundant genomic tools available and known genes underlying characters of interest, biotech approaches may facilitate breeding gains [42,158].

3. Conclusions

The criteria developed and presented herein are provided as a guide for ranking and screening species with the potential to become temperate-adapted, herbaceous perennial grain legumes suitable for mechanical harvest within commercial agriculture. Because plant domestication efforts should be initiated with a particular agricultural target in mind [34], some of the criteria may not be relevant for other agricultural settings (subsistence, tropical, and/or intercrop cropping systems that have commonly included trailing and vining grain legume species) even though they may be equally in need of new perennial grain legume species. Likewise, the few dozen species presented here within tables are not intended to represent the most promising or only species that merit initial consideration and evaluation, rather they serve as an example of how data for many of the criteria to be used in Phase I of the domestication pipeline can be acquired for some legumes using species monographs, looking at herbarium specimens and reading peer-reviewed literature. However, the size of the Fabaceae (more than 19,500 species) and its broad distribution across continents suggests that there are still many other potential candidates whose attributes are unknown and unavailable because they lacked previous agricultural interest or because their native regions have been underexplored.

Until recently, it was not even clear how many of the Fabaceae were herbaceous and perennial species, primary criteria for perennial grain legume candidates. To this end, a novel partnership uniting plant breeders, ethnobotanists and plant evolutionary biologists from The Land Institute, the Missouri Botanical Garden and Saint Louis University has been established to conduct a global inventory of perennial, herbaceous members of the Fabaceae, Asteraceae and Poaceae (Perennial Agriculture Project Global Inventory (PAPGI)). This ongoing project is intended to bridge knowledge gaps between botanical and agricultural research communities by compiling information originally collected by botanists for taxonomic, systematics and ethnobotanical purposes and making it accessible to breeders working to develop perennial grain crops through an online, searchable database (Ciotir et al., unpublished). Ultimately, the PAPGI will expand upon the work done here, offering an extensive accessible knowledge framework to support the development of novel, perennial grain crops from wild, previously undomesticated plant species for a wide variety of cropping systems and agricultural settings.

Lastly, Phase I of the pipeline strategy is not meant to be an exercise in simply gathering data about certain traits for potential species through database searches or empirical research; rather, it is also intended to be an evaluation that ranks and identifies species most likely to be successfully domesticated and grown as a crop. While surveys of important attributes can help narrow the list, previously unforeseen limitations and opportunities are likely to be revealed by simply planting, growing and harvesting seed from the candidates within agricultural settings [26]. Furthermore, because no species is expected to have all or even a handful of the required attributes, the time required to acquire the necessary traits or overcome known limitations via breeding is also unforeseeable. Breeding populations must be developed and selection must be performed for each species to identify heritable variation for crucial domestication traits, estimate response to selection for the traits and predict the rates of genetic gain (the domestication timeline) that can be expected. Therefore, only by growing the species and performing simple selection experiments can final decisions be made about which domesticates to drop from the pipeline and which to move forward to Phase II: Wild Species to New Crop. This approach represents a largely unexplored and rewarding area of potential research for breeders, evolutionary biologists and classical botanists alike.

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