



Restoring depleted Tunisian drylands with native steppic species: where should we source the seeds?

Marjolein Visser* and Dirk Reheul

Department of Plant Production, Faculty of Agricultural and Applied Biological Sciences, Coupure Links 653, 900 Gent, Belgium; *Author for correspondence (e-mail: Marjolein.Visser@rug.ac.be; phone: 32 (0) 9 264 60 99; fax: 32 (0) 264 62 24)

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Abstract

In many of the world's drylands genetic and soil erosion are widespread. To enhance success of any large scale restoration effort in these hazardous conditions, it is essential to set up systematic seed production of key native plant species. The aim of the traditional outline is to gather local yet ample genetic variation to avoid the risks of non-adaptation. But for *Stipa lagascae* R. and Sch, a North-African perennial bunch grass, we carried out an outline inspired from conventional forage breeding practice, with the aim to select for vigour. Here, we assess its outcomes as for the risk of selecting non-local and/or non-ample genetic variation. We established a collection composed of 276 clonal lines out of individuals coming from 70 accessions, spread over 6 major ecogeographic origins within Presaharian Tunisia (100 mm < average annual rainfall < 200 mm), both source and target region. During 7 successive growing seasons with stark contrasts for rainfall and fertility, we observed the collection for vigour and phenology. Principal Components Analysis has been carried out on a data matrix of 276 lines and 11 variables. Both for vigour and for phenology, individuals differentiated strongly, but irrespective of accession or ecogeographic origin. Moreover, differences for vigour persisted throughout contrasting growing seasons. Both facts combined (1) do not indicate any local adaptation within the target region, (2) suggest instead intense genetic structuring due to isolation by distance, (3) make it easy to cull a limited number of lines to combine considerable genetic variation with consistently superior vigour.

Introduction

Native seed production is an enterprise that is not only labour-intensive but also risky. If not well done it can flaw all restoration efforts that rely on it. One of the issues seed producers often overlook is genetic quality. Researchers routinely mention the risks of the use of seed sources ecologically unrelated to the restoration site (e.g. Montalvo et al. (1997), Gordon and Rice (1998), Knapp and Dyer (1998)). Native seeds should conserve a readily adapted potential for reintroduced populations to evolve: the genetic variation contained in the seeds should be *local, yet ample* and unmodified by erroneous seed production methods.

To achieve this, restorationists usually adhere to the principle of the *home site advantage* (Bennington and

McGraw 1995; Kindell et al. 1996). We call this approach Outline A for native seed production:

- A1. Look for seed sources as close to the restoration site as possible
- A2. Bulk seeds from as many plants as possible within these source sites
- A3. Do not screen for adaptive traits
- A4. Avoid to multiply seeds in radically different environments and limit the number of production cycles.

This approach is site-specific, and follows a central concept of ecological restoration: localness. However, in many cases it remains unclear to what extent (1) seed sources can be called local and (2) seeds from these sources indeed provide useful genetic variation.

If seed is bulk collected and reseeded, there will be an unassessable loss of genetic variation, typically because of differences in maturity and dormancy of the harvested seeds. In addition, the site specific approach is only appropriate as long as extensive seed sources of promising native species exist nearby. If the locality is genetically impoverished, a site-specific approach could easily prove dangerous (Havens 1998).

In contexts of widespread arid land degradation, such as in South-Tunisia, seed sources of promising native perennials have indeed become rare compared to the area that solicits restoration (Aronson et al. 1993a). In addition, reintroduction of native species has proved to be extremely hazardous, not only for technical but also for non-technical reasons, touching all aspects of society (Floret et al. 1989; Le Houérou 1991; Visser et al. 1997). In such complex contexts of continued genetic erosion, native seed production should pursue several objectives at a time. (1) Starter collections are to be set up for long term genetic conservation objectives. (2) Within these starter collections, selection of consistently superior material can considerably enhance restoration success within a broad target region. Keeping in mind that native seeds, whatever the approach, should ensure *local yet ample* genetic variation, we looked for an alternative, Outline B.

B1. Take as many individuals of as many accession sites as possible within recognizable ecological limits of the target region

B2. Install this material as a starter collection in a homogeneous rainfed site representative for the target region

B3. Select individuals that combine superior grazing value with diverse reproductive phenologies

B4. Multiply seeds of selected individuals in the best possible conditions

The procedure has been designed following a technical outline for the initiation of rapid seed production schemes of mostly outbreeding forage species in temperate climates (Tyler 1987), because promising native steppic species of South Tunisia are selectively grazed by domestic flocks, and will continue to be so in the foreseeable future. However, Outline B is not specific for arid regions, where rainfall is reputed for its extreme variability in space and time. Forage breeding programs generally do not work within this kind of extreme environments, not only because they are marginally productive for agriculture all together,

but also because breeders fear too important genotype by environment ($G \times E$) interactions, which would render the selection of consistently superior genotypes very difficult (Ceccarelli 1989; 1996). On the other hand, it has been argued that the temporal unpredictability inherent of marginal rainfall environments would select, amongst others, for high phenotypic plasticity, at least for annuals (Aronson et al. 1992; 1993b). High phenotypic plasticity would lead to relative stability of performance under a wide variety of climatic conditions, hence low $G \times E$ interactions, whether in space ($G \times$ location) or time ($G \times$ year), exactly what breeders are looking for. Unfortunately, candidate native seed producers for arid regions just do not know what to expect, because it has not been done yet. Until now, these supposed $G \times E$ interactions are mere speculations based on knowledge from less arid environments.

Outline B has been carried out in full for *Stipa lagascae* R. and Sch., a widely distributed yet threatened Mediterranean type bunch grass that is very promising for restoration purposes (Floret 1981; Waechter 1982; Chaieb et al. 1991; Aronson et al. 1993a; Le Floc'h et al. 1995). With a data set derived from this material, we address the following interrelated questions.

- Are individual differences in vigour and phenology within the starter collection of *Stipa lagascae* related to local adaptation or not?
- Is selection of *Stipa lagascae* within and for a marginal rainfall environment such as South-Tunisia feasible and sound, both from a breeder's (weak $G \times E$ interactions needed) as from a restorationist's (*local yet ample* genetic variation needed) perspective?

Material and methods

Target region (B1)

Seed production of *Stipa lagascae* targets Presaharian Tunisia in the sense of Floret and Pontanier (1982), which corresponds roughly with the Tunisian part of the lower arid zone of North-Africa, comprised between the isohyets of 100 and 200 mm of annual rainfall. It is part of the arid zone, where annual rainfall is between 100 and 400 mm, and which covers 11.10^6 ha, two thirds of Tunisia. Presaharian

Tunisia itself covers about 5–6.10⁶ ha. Rainfall pattern and temperature regime are Mediterranean.

Topographically, the entire Tunisian arid zone is composed of extensive sandy plains, formerly covered by *Rhanterium suaveolens* dominated steppes, and intersliced by low hill ranges and their more loamy foothills, formerly covered by *Artemisia herba-alba* dominated steppes (Le Houérou and Boulos 1991). Since the beginning of the 19th century, these dwarf shrub dominated rangelands have been transformed into an eroded patchwork of cereal cropland and olive orchards (both private land) interspersed with usually overgrazed steppe leftovers (common land or privatisation underway). The ecological range of most steppic species extends over the whole arid zone, but because the cultivation process has started much earlier in the northern part (towards the 400 mm limit, the theoretical minimum for permanent agriculture) they virtually do not occur anymore beyond the Presaharian part.

Soils are isohumic sierozems dating from the Miocene and lack horizon differentiation. The major feature of soil heterogeneity is the depth of the gypsoic crust under a relatively homogeneous sand-loam horizon. This variable depth is the prime limiting factor for both total water availability and root extension. Nutrient availability is always very low. In general, organic matter content < 0.6%, total N-content < 0.05%, 5 < Cation Exchange Capacity < 8 meq per 100 g (Floret and Pontanier 1982), Olsen-extractable P-content < 10 mg per kg (Ewing 1999) and 7 < soil-pH < 8 (Floret and Pontanier 1982; Ewing 1999).

Representative site (B2)

The site that was available for the starter collection is located in El Fje, 22 km east of Médenine and 5 km landward (33°30'N and 10°40'E). Its area is about 0.35 ha (50 × 70 m²) and its soil characteristics are considered as typical for the better soils of the Jeffara coastal plain of South-Tunisia, east of the Matmata mountains. A calcium-rich sandy-loam horizon overlays a gypsoic crust at a depth varying between 30 and 110 cm. This depth variation is partly due to a gentle NE–SW slope. Before installation of the starter collection, this field was regularly cultivated for extensive barley cropping, which explains the exceptional absence of stones, the destruction of surfacing

gypsoic crusts, and its invasion by *Cynodon dactylon* (Waechter 1979).

Installation and maintenance of the starter collection (B2)

In total we have carried out three collection campaigns to fill the starter collection site. For this paper we shall only focus on the clonal lines of the first one, which has been carried out during January, February and March 1993. Within each accession site, 2 to 8 individuals have been selected visually on vegetative vigour *in situ*. Small isolated clusters generally yielded 2–3 individuals but extensive populations in near-intact steppic vegetation yielded up to 8 individuals. We avoided to sample neighbours within accessions, and sites closer than 1 km apart between accessions. Transplanting occurred the day following collecting. Each individual was cloned into 10 transplants and planted as 1 clonal line. Lines of the same accession were grouped together. These lines have become the observation objects for their comparison. They were grouped into 8 units of 48 consecutive lines. Units are spaced 3 m apart, lines within units 1 m apart and transplants within lines 30 cm apart. A distance of 1 m is more than the average distance between nearest neighbours in steppe-like vegetations (McPherson 1997) hence lines are supposed not to compete for water.

In total, we have collected 359 individuals (cloned into 3590 transplants) from 72 accessions. Heavy losses occurred after transplantation: 23% of the lines and 66% of the transplants died before summer 1993. These losses imply that at the end of the growing season 1992–1993 only 276 lines from 70 accessions (Table 1) survived with on average 4.4 transplants and a minimum of 1 transplant per line. These 276 lines are dispersed over 8 contiguous units initially planted. However, after this first year losses have been minimal. So these surviving clones, although heterogeneously spaced, could be continuously evaluated from 1993 to 1999 (7 successive growing seasons). The gaps of completely lost lines have been filled with lines from the two following collection campaigns.

The total collection site has been kept weed-free with a small rotating-disk cultivator between lines and by hand within lines. During the growing season, locally abundant swards of *Cynodon dactylon* were regularly eradicated with glyphosate. Once a year,

Table 1. Grouping of 276 individuals (#ind) and 70 accessions (#acc) of *Stipa lagascae* into 6 ecogeographic origins. Origins are named according to a nearby reference point and are distinguished on climatic transition zones, existence of relief, degree of fragmentation and human habitation (always in that order). The spread of 40 individuals of a rough and a fine-tuned selection for vigour over different accessions and ecogeographic origins is given in the last column.

Origin	Nearby reference		Key features	Starter		Selection				
	Name	Coordinates		collection		Rough		Fine-tuned		
		Lat		Long	#ind	#acc	#ind	#acc	#ind	#acc
1	Taguelmit	32°58'	11°18'	Lower arid and transition to upper saharian; no relief; almost unfragmented range; not inhabited	82	16	16	10	13	9
2	Médenine	33°20'	10°29'	Lower arid; no relief heavily fragmented; inhabited	73	20	8	7	7	5
3	Mezzouna	34°34'	09°51'	Lower arid near transition to upper arid; no relief; heavily fragmented; inhabited	13	5	1	1	2	2
4	Tamezret	33°32'	09°52'	Upper saharian west of Tamezret; relief; slightly fragmented; not inhabited	17	7	5	4	4	4
5	Tataouine	32°55'	10°26'	Transition lower arid to upper saharian south-east of Tataouine; relief; heavily fragmented; inhabited	45	10	5	3	6	5
6	Sidi Touï	32°43'	11°22'	Upper saharian; relief, National Park; not fragmented; not inhabited	46	12	5	5	8	6
TOTALS					276	70	40	30	40	31

after seed dispersal, all lines were cut to the ground. The lines of the 1992–1993 campaign have only been irrigated during the installation year. They have been fertilised twice with N (50 kg ha⁻¹) and P (100 kg ha⁻¹) at the start of the two growing seasons following installation (1993–1994 and 1994–1995). In Jan 1999 they have been fertilised again with N (25 kg ha⁻¹). All fertilisers have been incorporated near the tufts when the soil was moist.

Data collection

Vigour

Vegetative vigour, hence grazing value, is routinely evaluated through measures of dry herbage yield and, simultaneously or alternatively, through visual scoring of the apparent leaf mass (Hyder 1972; Tyler 1987; Fily and Balent 1991; Boonman 1993). Because of the heterogeneity of the lines of the starter collection of *Stipa lagascae*, only visual scoring was feasible. The scorings have always been carried out by the same observer, using a scale from 1 (not vigorous) to 5 (very vigorous). This has been done between 2 and 10 times per growing season throughout 7 successive growing seasons (Table 2). Calculation of arithmetic year averages of these visual scores

yielded a time series of 7 successive year scores per line (TOT93 to TOT99).

Phenology and reproductive strategies

When annual rainfall exceeds 250 mm, water availability is not limiting anymore for plant growth in Presaharian Tunisia (Chaieb 1989). During the exceptionally wet growing season of 1995–1996 (356 mm of well distributed annual rainfall, Table 2), the lines of the starter collection showed maximal differentiation in all aspects, hence also for phenology. It was the only growing season of which we can reasonably accept that interaction between phenology and transplant number per line was minimal. The switch from vegetative to generative growth occurred from late December 1995 onward. Floral initiation and following phenophases have been recorded repeatedly with visual scores by the same observer. Close study of the floral initiation of dominant tillers needs regular apex cuts under binoculars, but the switch goes along with synchronic changes of the outward tiller aspect: appearance of third and last vegetative leaf, tiller swelling and elongation of leaf sheaths (not yet of the culm). At two dates during the first half of January 1996 (02.01 and 14.01), floral initiation of the dominant tillers as observed by these changes of tiller aspect has been scored using a binary system (0 for

Table 2. Features of 7 successive growing seasons and Spearman correlation coefficients between corresponding mean year scores for vigour (TOT93–TOT99), planting date converted to day of the year (DATE), number of surviving transplants (PLANT), the sum of scores for floral initiation (SUMINIT) and the sum of scores for developmental rate (SUMPHEN) on 276 individuals of *Stipa lagascae*.

Main growing season features				92–93	93–94	94–95	95–96	96–97	97–98	98–99	
Rainfall Mm.year-1				82	173	153	356	74	145	295	
Month of onset				Dec	Nov	Oct	Sep	Jan	Sep	Oct	
Number of scores on vigour				2	4	6	9	10	3	7	
Spearman correlation coefficients											
	Date	Plant	Sumnit	Sumphen	TOT93	TOT94	TOT95	TOT96	TOT97	TOT98	TOT99
Date	1	–0.351	0.120	0.047	–0.560	–0.501	–0.017	–0.109	0.270	0.172	0.059
<i>P-level</i>		0.000	0.047	0.435	0.000	0.000	0.783	0.070	0.000	0.004	0.333
Plant		1	0.065	0.109	0.605	–0.514	0.212	0.418	–0.008	0.160	0.167
<i>P-level</i>			0.283	0.072	0.000	0.000	0.000	0.000	0.901	0.008	0.006
Suminit			1	0.791	–0.086	0.000	0.123	0.117	0.066	0.036	0.100
<i>P-level</i>				0.000	0.155	0.999	0.042	0.051	0.273	0.557	0.098
Sumphen				1	–0.011	0.107	0.159	0.215	0.060	0.020	0.149
<i>P-level</i>					0.857	0.077	0.008	0.000	0.322	0.735	0.014
TOT93					1	0.781	0.150	0.205	–0.170	–0.095	–0.047
<i>P-level</i>						0.000	0.013	0.001	0.005	0.117	0.442
TOT94						1	0.298	0.318	–0.018	0.021	0.079
<i>P-level</i>							0.000	0.000	0.765	0.730	0.191
TOT95							1	0.620	0.454	0.331	0.278
<i>P-level</i>								0.000	0.000	0.000	0.000
TOT96								1	0.580	0.471	0.388
<i>P-level</i>									0.000	0.000	0.000
TOT97									1	0.623	0.451
<i>P-level</i>										0.000	0.000
TOT98										1	0.719
<i>P-level</i>											0.000
TOT99											1

vegetative apices and 1 for generative apices). According to the sum of these two scores (SUMINIT) lines were classes as early (sum = 2), intermediate (sum = 1) or late (sum = 0) for floral initiation. From the moment of general floral initiation (end of January 1996) until early seed dispersal (end of March 1996), lines have been scored 5 times (on 22.01, 14.02, 29.02, 11.03 and 29.03) on flowering stage using a scale from 1 to 8. A score of 1 corresponds to completed culm elongation but still closed floral leaf sheath, 3 with the first awned spikelets piercing through the floral leaf sheath (heading), 5 with full inflorescence emergence and anthesis, and 8 with full seed dispersal. These scores were given by averaging flowering stage visually over the whole line. According to the sum of these 5 scores (SUMPHEN), lines were classed as slow (SUMPHEN < 10), intermediate (10 ≤ SUMPHEN < 20) or fast (SUMPHEN ≥ 20) with regard to their developmental rate. The limits 10 and 20 between these classes correspond to the mean ± standard deviation over all 276 sums. Both classifications were used for selection purposes later on.

Data analysis

Spearman rank coefficients have been calculated between planting date (dates transformed to day of the year, DATE), transplant number (PLANT), year scores for vigour (TOT93 to TOT99), SUMINIT and SUMPHEN. On the Pearson correlation matrix of the same set of variables, Principal Components Analysis (PCA) has been carried out. To assess possible group differences, the 276 individuals of 70 accessions have been grouped into 6 distinct ecogeographic origins, according to climate and current fragmentation of the original plant cover (Table 1).

Results

Contrasting growing seasons

Rainfall patterns from 1992–1993 to 1998–99 have been extremely contrasting in terms of total rainfall as well as distribution. Table 2 shows rainfall totals as well as months in which the opening rains fell. The

late onset of the rains (mid-December) in the installation year 1992–1993 explains why prospectations have started only in January. However, the 1992–1993 drought is not relevant for vegetative vigour of the transplants because they have been irrigated. From the summer of 1993 onward the transplants have been rainfed. 1995–1996 was exceptionally wet and offered a long growing season. Rains started already in September. The following growing season 1996–1997, was as exceptionally dry as 1995–1996 was

wet. In addition, the opening rains fell only in January. Most perennials of the steppe, including *Stipa lagascae*, did not flower. Rainfall of 1998–1999 was much above average but not well distributed; the opening rains (122 mm at a time) fell only end October. The other years are near-averages for El Fje.

Vigour, phenology and the influence of transplantation

The first three principal components (PC1, PC2 and PC3) resulting from the PCA explain 70% of the total variation (30, 24 and 16% respectively). Figure 1 shows the loadings of all variables on those components and a loading plot in the plane (PC1, PC2). Three groups of successive year scores appear: (1) TOT93–TOT94, strongly positively correlated with transplant number (PLANT) and negatively correlated with transplant date (DATE), (2) TOT95–TOT96, with the highest loadings for PC1 and (3) TOT97–TOT99. There are however no significant negative correlations between any pair of year scores (Table 2).

Tuft survival (PLANT) was clearly affected by planting date (DATE). As the planting season of 1992–1993 advanced, more lines failed completely (data not shown). For the 276 surviving lines, late planting resulted in a reduction of the final number of surviving transplants. However, late floral initiation and/or slow developmental rate did not explicitly favour transplant survival of lately transplanted lines, as neither SUMINIT nor SUMPHEN are correlated with PLANT or DATE. The influence of the planting date on vigour stretches far beyond 1992–1993, for the correlation between vegetative vigour and planting date only ceases to be significantly negative from 1994–1995 on. The stretch of this influence corresponds to the considerable switch from TOT93–TOT94 to TOT95–TOT96. So only from 1994–1995 on, the true vigour of part of the lines could fully express. Most, but not all of these belong to late planted accessions. Since then, despite enormous rainfall and fertility differences, correlations among all possible pairs of year scores (TOT95–TOT99) remain highly significant, and these variables also have the highest loadings on PC1. There is however a slighter switch between TOT95–TOT96 and TOT97–TOT99 for which several factors account.

According to the Spearman correlation matrix (Table 2) and PC3 SUMINIT and SUMPHEN are strongly correlated with one another. On the other

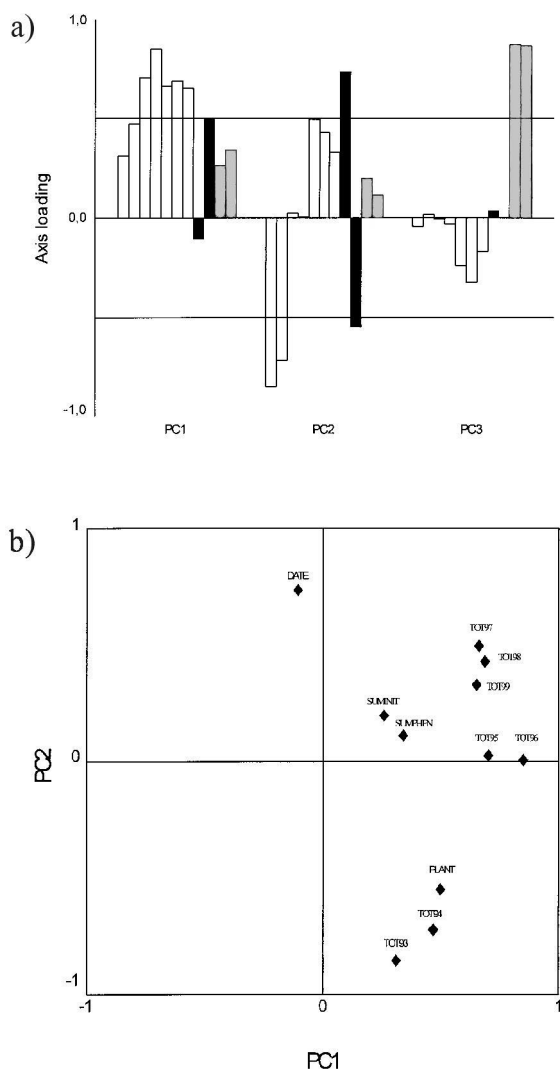


Figure 1. a and b. (a) Loading scores of the 11 initial variables on the first 3 principal components (PC1, PC2 and PC3) resulting from PCA. These variables are invariably plotted in the following order: TOT93–TOT99 (white bars), DATE, PLANT (black bars) and SUMINIT, SUMPHEN (grey bars). (b) Loading plot of the same variables in the plane (PC1, PC2).

hand, PC1 and PC2 both combine high positive loadings of TOT95–96 with positive loadings of SUMINIT and SUMPHEN, whereas PC3 combines high positive loadings for SUMINIT and SUMPHEN with negative loadings for TOT97–TOT99 (Figure 1a). Hence, a slight shift in correlation between vegetative and reproductive development (from positive to negative) has accompanied the shift in position of year scores from TOT95–TOT96 to TOT97–TOT99. We attribute this to a masting effect for part of the collection (Haase et al. 1995). Those vigorous lines that were at the same time early initiators and rapid developers, responded to the prolific growth-conditions during 1995–1996 with massive inflorescence production, exhausting any remaining vegetative tillers, which left them with less vegetative vigour during the following years. This phenomenon was possibly exacerbated by the severe drought of 1996–1997, and by the ending effect of the P-fertilisation in 1993–1994 and in 1994–1995. Adequate available P (above the absolute minimum of 10 mg per kg) indeed enhances water and N-uptake, but on CaCO_3 -rich soil P-immobilisation makes that the fertiliser effect cannot stretch beyond 1996 (Matar et al. 1992).

This second switch of vigour, which is a $G \times \text{Year}$ interaction, relates to a minority of lines. The main factor explaining the variation remains PC1: it explains 30% of all variation and has high loadings for all year scores after 1994. If we omit DATE, PLANT, SUMINIT, SUMPHEN and TOT93–TOT94 from the dataset and re-analyse TOT95–TOT99 for the same 276 individuals, the first PC1 accounts for 61% of the total variation and has high loadings for all remaining year scores. So despite variation in rainfall and soil fertility, and despite masting and alternancy, the bulk of the collection shows consistency in vigour once the effect of transplantation is leveled out.

Individual vigour differences and rough culling (B3)

Figure 2 shows the differentiation of all 276 lines according to their scores for the PC1 (mainly accounting for vigour from 1994–1995 on) and PC3 (mainly accounting for phenology). Markers indicate their ecogeographic origins. Outline B3 says first of all that individuals with superior grazing value are to be selected. Typically, from a forage breeder's perspective, the individuals with the highest scores for PC1 would be selected for consistently superior vigour. The first 40 individuals have a score above 1 for PC1

(mean = 1.547 and minimum = 1,014), exhibit widely different phenologies, belong to all 6 ecogeographic origins, and to 30 different accessions (Table 1). This is, only some accessions have contributed more than 1 individual to the cull. However, the proportions of the 6 origins among this cull have changed. Origin 1 (Taguelmit) and 4 (Tamezret) yield disproportionately more individuals to the cull at the expense of the other origins, including Origin 2 (Médenine), to which the site of the starter collection belongs.

Reproductive strategies and fine-tuned culling (B3)

More than the resulting score for PC3, the classification for both floral initiation and developmental rate helps to retrieve individuals with extreme reproductive strategies. The combination of both classifications yields 3×3 phenological classes (Figure 3). The 3 classes according to floral initiation (early, intermediate and late) are equally represented (29, 30 and 41% of the 276 lines respectively). Only the early initiators have a well represented class of fast development (16%) and only the late initiators have a well represented class of slow development (11%). This classification is what one might expect. With regard to floral initiation the lines differentiate well, but the differences in length of reproductive period are smoothed out by the overriding influence of rising spring temperatures during heading. Among the 40 most vigorous individuals ($\text{PC1} > 1$), the proportion

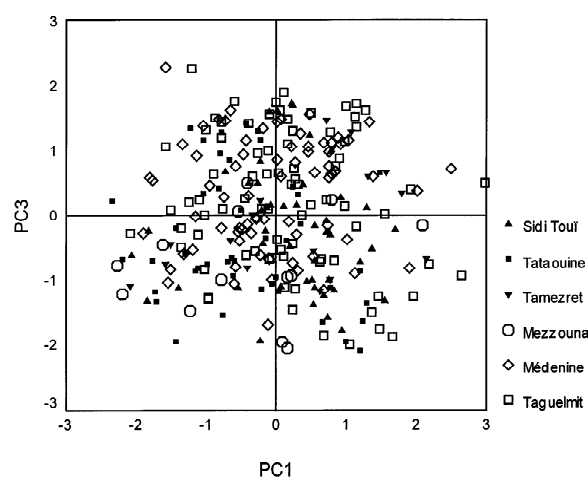


Figure 2. Factor score plot in the plane (PC1, PC3) of 276 individuals of *Stipa lagascae*. Markers represent 6 ecogeographic origins (see Table 1). PC1 mainly accounts for vigour, PC3 for timing of floral initiation and developmental rate (cf. Figure 1a).

of early initiators and fast developers (class 1) has almost doubled at the expense of the proportion of late initiators (classes 8 and 9) or rare combinations, which are not present at all (class 4 and 6). This is not balanced from the restorationist's perspective. Therefore, this rather rough cull could be fine-tuned by (1) including the condition of proportionate representation of all reproductive strategies compared to the whole starter collection, (2) accepting only several individuals from the same accession if they belong to different phenological classes. In this case, the fine-tuning reduced the mean score for PC1 of the cull to 1.460 (minimum = 0.656), diminished somewhat the dominance of Origin 1 (Taguelmit), but did not really augment the number of accessions (Table 1). This means that accessions do not only host very different genotypes with regard to vigour, but also with regard to phenology.

Discussion

Is adaptive genetic variation always the result of local adaptation?

Outlines A en B for native seed production clearly use the concept of genetic structure within plant populations in different ways. Genetic structure within and among plant populations can be explained by spatial

environmental variation (hence local adaptation through 'natural' selection) and by isolation by distance. Besides this, much genetic variation necessarily remains unexplained, because genetic structure is rarely a steady state equilibrium (Heywood 1991). Instead of local adaptation, it could very well reflect residual patterns of unknown historical events, essentially sudden biotic or abiotic changes of the environment, or unusual diaspore or pollen migrations, many of which could have been man made.

Adaptation and ecotypic differentiation have been widely documented in ecological research, using a number of adaptive traits that determine reproductive fitness. If allowed enough time, aridity should enhance this, because growth conditions vary tremendously in space, and are likely to favour local adaptation, ecotypic differentiation, hence $G \times E$ interactions (e.g. Theunissen et al. (1992), Theunissen (1995, 1997)). The idea that genetic variation occurs mainly through local adaptation, at equilibrium with unchanged selective forces, has fed the principle of the home site advantage, and the site specific approach of Outline A.

On the other hand, the study of genetic variation through isolation by distance is much more recent. Using molecular polymorphisms with genetic or enzymatic markers of a given species (e.g. Schuster et al. (1994)), these studies forward several explaining variables such as breeding system, longevity, timing

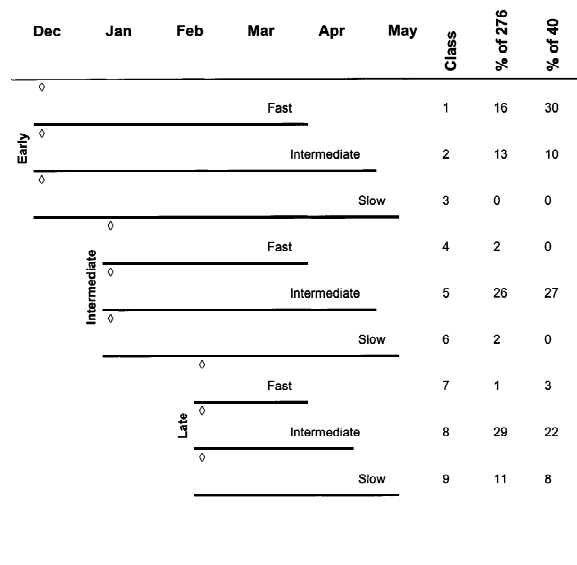


Figure 3. Classes for timing of floral initiation (early, intermediate and late) and for developmental rate (fast, intermediate, slow). Time indications schematically illustrate relative class differences. The last columns show % representation of these classes among 276 individuals of *Stipa lagascae* and among a rough cull for vigour (40 individuals).

of reproduction, successional stage, geographic range, and population density, that can predict the partitioning of genetic variation of these molecular markers within vs. among populations (Loveless and Hamrick 1984; Godt and Hamrick 1998). However, because polymorphisms are neutral toward selection, hence not adaptive, they are of little value for restorationists who wish to assess the risk of non-adaptation of native seeds (Knapp and Rice 1997). Obviously, also the study of adaptive genetic variation through isolation by distance is important for native seed producers, because it would help them to assess the risk of 'going too local', which would result in too little genetic variation within native seeds. Nevertheless, this kind of studies is labour-intensive: it would require an individual approach and extensive sampling within and among populations, so ecologists rarely carry them out.

Intriguingly, breeding strategies (an example of which is Outline B), use the idea of local adaptation only in a very broad sense (Ceccarelli 1989): the restorationist's home site thus becomes the breeder's target region. Because of all the uncertainties associated with its real causes within this target region, plant breeders consider genetic variation as a black box. Plant breeders do not try to explain the genetic variation they uncover, although they explicitly need it to find widely adapted genotypes. To achieve this, the focus on individuals through large starter collections is typical. They look for extreme individuals in a range of genetic variation that should be as large and as easy to detect as possible: not levelled out by bulking accessions, not masked by environmental differences between accession sites nor by sampling biases and, above all, adaptive. In fact, they carry out what ecologists rarely carry out: large-scale studies of adaptive variation within and among populations, without really describing the outcomes, concentrated as they are on 'keep the best and throw away the rest'.

The weak point in whatever breeding strategy is an ecologically sound definition of the target region. If too broadly defined, $G \times E$ interactions (alternatively called local adaptation) become too important and render selection of consistently superior genotypes impossible. As said, arid regions are very particular and under-researched in this sense.

Differentiation of Stipa lagascae: individuals, accessions and ecogeographic origins

In the case of *Stipa lagascae*, once the effects of late

transplantation were overcome, following constants emerged:

- Almost all transplants and all lines survived 7 contrasting growing seasons. So no differentiation between accessions or ecogeographic origin occurred with respect to mortality.
- Differentiation for vigour did not occur according to ecogeographic origin and was almost unrelated to environmental variation in time, although this variation was extreme in terms of water and nutrient availability. Differentiation for floral initiation and developmental rate did not follow a pattern according to ecogeographic origin either, and was overall uncorrelated with vegetative vigour.
- Throughout time, consistently vigorous lines appeared, although they originated from widely different accessions and ecogeographic origins.
- Although very few individuals were sampled within each accession site, those individuals showed to differentiate very well with regard to vigour and phenology.

These constants do not suggest any local adaptation on any scale smaller than that of Presaharian Tunisia. Even accessions do not behave as homogeneous units. This is, the 'home site' is indeed equivalent to the target region itself. It follows directly that, if the target region is correctly delimited, $G \times E$ interactions are not prohibitive for selection in arid environments. Our data further suggest that, within the target region, genetic variation of *Stipa lagascae* is more due to isolation by distance than to environmental heterogeneity. We can further argue this with the following facts.

Stipa lagascae combines cleistogamy, longevity and low population density. This combination comes back in several other North-African candidate species for native seed production. Furthermore, within the steppes for which these species are representative, recruitment events of perennials are extremely rare, as are mechanisms for long range seed dispersal (Ellner and Shmida 1981; O'Connor 1991; Haase et al. 1995). All these variables combined favour fine-grained genetic structuring through isolation by distance, and hamper the process of local adaptation (Loveless and Hamrick 1984; Godt and Hamrick 1998). The seeds of *Stipa lagascae* are heavy and have long awns. Unaided seed dispersal should therefore be limited to the immediate environment of the mother plant (Ghermandi 1995). However, its seeds are also very sharp and get easily stuck in cloth, wool

and skin. Given the semi-nomadic nature of the human populations and their domestic flocks, who crossed both administrative and ecogeographic boundaries until some decades ago (Louis 1979), it is very likely that frequent long range transport has favoured recurrent migrations to more or less remote places. However, these migrations could only become effective if recruitment and survival of seedlings followed. This combination of events seems quite unlikely (O'Connor 1991). But if these assisted migrations were effective even just a few times, they would have created additional genetic variation unexplained by any local adaptation process.

Bulk (Outline A) vs. individual (Outline B) approach

As for the issue of genetic variation vs. selection, our experience suggests some practical advice. Restorationists working with a broadened home site concept, could argue that all the genetic variation of the starter collection should be present in the seed multiplication plots (stages A4 and B4 of Outlines A and B). However, bulk seed multiplication is always hazardous because of unpredictable and unnoticeable genetic shifts, especially when the multiplication environment is radically different from the target environment (Knapp and Dyer 1998). To avoid this, the seeds of individual genotypes could be multiplied separately. This is obviously impossible to realise with all the lines of the starter collection. It follows that a cull of a manageable yet not too small number of lines is unavoidable. It is very plausible, but impossible to prove, that the historical overexploitation of the steppe, and concomitant decline of soil fertility, has favoured the disappearance of resource-responsive species and genotypes within species. So why not a cull for vigour, if this would add success to restoration projects? Our results with *Stipa lagascae* show that, within a cull for vigour of 15 to 20% of the original number of lines, genetic variation is still considerable, but already biased toward certain phenological classes and ecogeographic origins. As already shown, fine-tuning by imposing some boundary conditions does not seriously affect the overall vigour of the cull. The larger the starter collection, the larger the variation, the more comfortably native seed breeders can cull for vigour, and fine-tune later on, to counterbalance possible biases in phenology and ecogeographic representation. If lines are multiplied separately, seed mixture compositions can be changed at will, for example to equalise founder representation

(Havens 1998), or because the selection strategy has incorporated additional parameters (dormancy patterns are likely concern), or for particular restoration targets.

Can similar selection strategies be used for other candidate species of the North-African arid belt? B1 and B2 can be easily applied to other species, because ecological boundaries for target regions within the North-African arid belt are quite well described. But the details of B3 and B4 depend on species-specific variables and what kind of genetic variation is revealed by the starter collection. We suspect that the majority of other candidate species exhibits a similar fine-grained genetic structure due to the cited combination of ecological variables. However, accurate knowledge about breeding systems, seed and pollen dispersal mechanisms, and genetic variation lacks for the great majority of North-African native species. The two-layered approach (individuals/accessions) during the set up of starter collections is extremely helpful to acquire at least some basic insight into those unknowns. Furthermore, the focus on individuals could become less laborious if these were collected by seed (less work, losses and limits imposed by the short planting season). But collectors should be aware that seed collecting gives rise to other problems, such as seed dormancy, long juvenile phases in case of woody species, heterogeneous progenies in case of allogamous species, or unconscious selection of outspoken seed producers (Tyler 1987). With a representative starter collection, and once the basic knowledge is acquired, native seed producers have tools to construct a selection strategy best suited for a particular species. For this matter we suggest to wait for at least one year with abundant rainfall before taking any crucial decisions. Finally, the relevance of either native seed production strategy should be evaluated by monitoring the behaviour of released plant material. Reseeding of degraded land provides opportunities to design sound and simple experiments to generate useful information. In this respect, restorationists should consider that selected plant material will best differentiate from non-selected material when soil fertility is restored and maintained through sound arid land use, the combination of which they ultimately seek.

Conclusion

We suggest that, at least for *Stipa lagascae*, but probably for other North African candidate species,

the alternative native seed production strategy (Outline B) is realistic, and can be made ecologically sound, even for extreme environments, if the target region is correctly delimited. Nevertheless, if Outline B is judged too costly, Outline A could still be applied if the concept of the 'home site' is broadened. More in general, these results also show that, whenever ecological restoration, or the prospect thereof, needs a regular supply of native seeds for a few key-species, Outline B could be just another way of dealing with localness.

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