

Improving pea production – Yield and nitrogen content of pea cultivars with different leaf types

Dissertation

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Dedicated to my parents and my wife.

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Eidesstattliche Erklärung

Ich erkläre an Eides statt, dass ich die Arbeit selbstständig und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Alle Stellen, die wörtlich oder sinngemäß aus Veröffentlichungen oder anderen Quellen entnommen sind, sind als solche kenntlich gemacht.

Göttingen, 4 April 2022



A handwritten signature in green ink, appearing to read 'S. Thauler', is written over a horizontal line.

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Chapter 1

General Introduction

1.1 The pea crop

Pea (*Pisum sativum* L.) is one of the oldest domesticated crops in the world, and it is the third most widely grown legume after soy bean and common bean. Its seed serves as a protein-rich source of food and feed (Smýkal et al., 2011). The very beginning of the cultivation of pea is considered in Near East from the early Neolithic (7000 to 6000 B.C.). Peas then appeared in the Neolithic agriculture settlements in Europe, accompanied by the production of wheat and barley (Zohary & Hopf, 1973). Zohary and Hopf also mentioned that cultivated pea varieties are distinguished from wild peas by the characteristics of the surface of the seed coat, which is smooth rather than rough or granular.

Pea is a diploid ($2n = 14$ chromosomes) and predominantly self-pollinated crop. Domesticated pea shows a wide range of morphological variation. Therefore, it is well adapted to both warm Mediterranean-type and cool temperate conditions. In peasant communities in Southwest Asia, the Mediterranean Basin, temperate Europe, Ethiopia, and north-western India, it constitutes an important source of protein for human consumption (Weiss & Zohary, 2011).

Pea has many uses: immature pods and seeds are used as green vegetables, either fresh or frozen; the whole plant is used as green forage for grazing animals, fresh or as hay or silage; and it is grown for its mature dry seeds, which have many uses (French, 2016).

Most cultivated peas are used in the form of dry seed and as supplements to human diets, mainly in the form of split peas for soup or pottage, or as canned products and pea also offers a bulk source of seed protein for man and animals with a relatively short growing season when compared with other legumes (Savage & Deo, 1989).

Peas have long been recognised as an inexpensive, readily available source of protein, complex carbohydrates, vitamins and minerals for human and animal consumption (Dahl et al., 2012).

1.2 The role of pea in the cultivation system

Pea ranks among the world's most important pulses (Smartt, 1990; Davies, 1995 in Weiss & Zohary, 2011). Pea is the most important cool season legume in Europe, though the harvested area and production of soybean are almost double the amount of pea. World production of peas in 2019 was about 14 million tonnes, the major producing countries being Canada, Russian Federation, China and the USA. The world area harvested of dry pea was above 7 million ha, with over 800.000 ha in the European Union (FAO, 2019).

Today, pea is grown as a component of cropping systems due to its many benefits, such as reduce nitrogen fertilizers through the ability of fixing N from the atmosphere in symbiosis with *Rhizobium* bacteria that is left in the soil and then can contribute to the productivity of the following crops (French, 2016). It can also be grown in mixture with cereal crops for reduce weed abundance, improving lodging resistance and increase the productivity of the crops compared to monoculture stands (Rauber et al., 2001; Bedoussac et al., 2015; Carlsson et al., 2017).

In organic farming, synthetic fertilizer is rejected. Therefore, pea and other legumes can be considered as a crucial component in the crop rotation by the ability of providing nitrogen to the organic system for the succeeding non-leguminous crops via symbiosis with N fixing bacteria (Gollner et al., 2019).

1.3 Leaf types in pea

Similar to other legume species which have the compound leaves, pea also has its pinnately compound leaf consisting of basal, foliaceous stipules, a rachis supporting some opposite pairs of proximal leaflets and distal tendrils (Young, 1983; Gould et al., 1994; Yaxley et al., 2001; Lewis et al., 2005; Hofer et al., 2009) (Figure 1a).

Regarding the morphology of the pea leaf, the characteristics of leaflets and tendrils are usually the main consideration (Young, 1983; Gourlay et al., 2000), and some researches concentrated also on stipules as well (Lafond et al., 1981; Côté & Grodzinski, 1999). However, in this study we concentrate on leaflets and tendrils as the plant type of almost all cultivars in production nowadays can be classified with these two characteristics.

The development of leaflets is determined by the *AFILA* (*AF*) gene which was described as a mutation by Kujala (1953). *AF* gene affects pinna (leaflet) length and pinna branching in

addition to pinna type, and regulates the timing and direction of leaf developmental processes in the proximal region of the leaf, but has little effect on the distal region. On the other hand, the *afila* (*af*) mutation causes leaflets to be replaced by compound (branched) tendrils (Villani & Demason, 1999). *AF* is completely dominant over *af* in the proximal region, but it is incompletely dominant in the distal region (DeMason & Villani 2001).

The forming of tendrils is controlled by *TENDRIL-LESS* (*TL*) which was discovered by Vilmorin & Bateson (1911). The dominant allele *TL* is in charge of forming tendrils, which is observed in wild type with homozygous *TLTL* allele. In reverse, the *tendrill-less* (*tl*) mutation (recessive allele) transforms tendrils into leaflets. The homozygous *tltl* genotype displays the disappearance of tendrils, while the heterozygous genotype (*TLtl*) of the semi-dominant mutation expresses narrow, subterminal leaflets in place of tendrils (Hofer et al., 2009).

The combination of these two genes (*AFILA* and *TENDRIL-LESS*) with both dominant and recessive alleles was described in detail by DeMason & Villani (2001) in about seven genotypes. However, our research materials just included two different leaf types of normal-leaved (*AfAf TLTL*) and semi-leafless (*afaf TLTL*) (stipules still exist, but the leaflets are developed into tendrils).

Based on the combination of *AFILA* and *TENDRIL-LESS* genes, Mikić et al. (2011) mentioned four main phenotypes (Figure 1.1.b, 1.1.c, 1.1.d, 1.1.e) which are (1) wild type (*AFAF TLTL*), with two or three pairs of leaflets and numerous terminal tendrils; (2) ‘*afila*’ (*afaf TLTL*), with tendrils only; (3) ‘*clavicula*’ (*AFAF tltl*), with leaflets only; (4) ‘*pleofila*’ (*afaf tltl*), with rachids ending with small leaflets.

The two leaf types in the field are shown in the Figure 1.2.

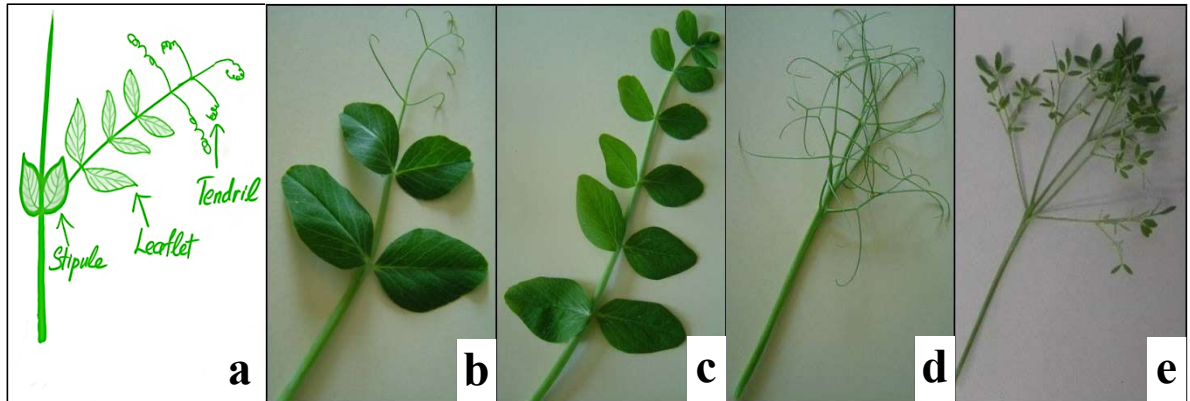


Figure 1.1 a) Leaf base and stipules of pea, and four basic leaf types of pea, regarding leaflets and tendrils: b) wild type (AFAF TLTL), c) tendril-less (AFAF tltl), d) afila (afaf TLTL), e) afila-tendril-less (afaf tltl) (Mikić et al., 2011).

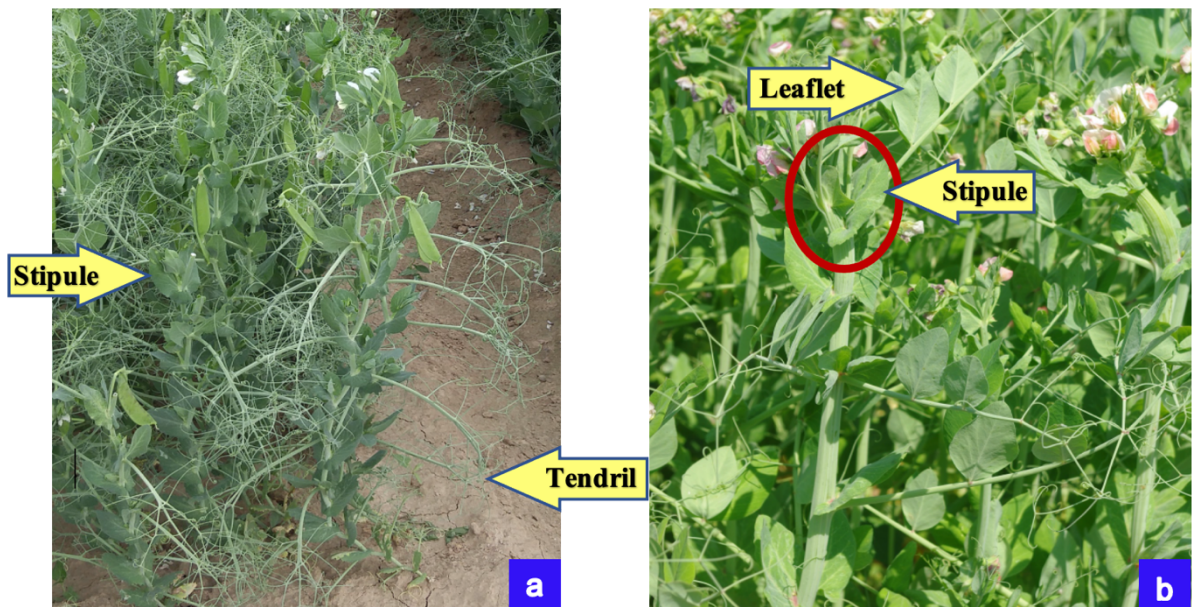


Figure 1.2 The performance of a) semi-leafless pea and b) normal-leaved pea in the 2018 field experiments in Reinshof, Göttingen, Germany.

A major problem associated with the pea crop is its tendency to lodge or its lack of standing ability. The pea plant is a natural scrambler and its long vines and tendrils make it ideally suited to growing through other vegetation. One of the traditional ways of growing peas is against support either in the form of small branches or twigs or against wires. Grown as a monoculture without support the planting density is such that neighbouring plants become attached to each other within the canopy. This may keep the crop standing for some time, but the canopy often collapses with the weight of pods and seeds as the crop matures. This

greatly impedes harvesting and creates an ideal micro-climate for fungal diseases (Ambrose, 2008).

Normal-leafed pea cultivars in monoculture system often show serious lodging right after flowering (Stelling, 1997), and this will cause a large yield decrease (Stelling, 1994). This reduction is due to the large amount of foliage of normal-leafed varieties that shades lower plant parts and results in decreased light penetration, photosynthetic activity of the lower leaves, and potential build up of pathogens in the moist lower canopy environment (Meadley & Milbourn, 1971).

The disadvantages of normal-leafed type / wild type raised the requirement for the new plant type which could improve the standing ability of pea. In 1970s, the *afila* mutation was applied to develop the fully leafless (*stst afaf*, in which *stst* is a mutation of *STST*, which significantly reduced the size of normal stipules in wild type peas) ideotype in a pea breeding program by John Innes Institute, and the first UK ‘leafless’ genotype names Filby was released in 1978 (Ambrose 2004 in Mikic et al. 2011). However, this ideotype of pea was unable to produce sufficient photosynthate at low planting densities through a limitation of the total biomass. In an attempt to find a new plant type which suite the mechanised harvest, the use of *AF* mutation with retained wild type stipules led to the development of semi-leafless cultivars which improved photosynthetic capacity, but still have the characteristic of lodging resistance.

Great improvements have been made in the standing ability of pea cultivars over the past 30 years. This is due to improvements in stem strength, shorter internodes, and the use of the semi-leafless (*afila*) character. Semi-leafless peas have more interlocking tendrils than conventional leafed plants and therefore forms a more rigid canopy framework than conventional pea. These canopies are better able to withstand the wind-imposed shearing forces that normally cause pea canopies to lodge. Today, nearly all European and North American pea cultivars are semi-leafless, and semi-leafless cultivars with improved stemstrength have become dominant also in Australia over the past decade (French, 2016).

Although, semi-leafless peas are more resistance to lodging, their protein content was sometimes reported to be lower than that of the normal-leafed peas (Čupić et al., 2013; Lietzow et al., 2013; Quendt et al., 2014; Olle, 2017). In addition, Simioniuc et al. (2002) reported that within the semi-leafless group large differences in the lodging score can be found between cultivars of the same height, e.g. between “Eiffel” and “Graffila”.

For organic farms in dry regions, Gollner et al. (2019) suggested that normal-leafed pea types should be favored because of their higher shading ability, and therefore better competitiveness against weeds. Various pea breeding programs and studies which determined the pea ideotype or cultivation method suitable for organic farming or low-input system has been performed (Annicchiarico & Filippi, 2007; Syrový et al., 2015; Georgieva, 2017).

There are some previous studies comparing the performance of two main leaf types in production. However, those researches just were conducted with a limited of material, and the results illustrated inconsistency of the performance of each leaf type. Some studies concluded semi-leafless cultivars gave higher seed yield than normal-leafed genotypes (Narits, 2008 in Olle 2017; Acikgoz et al., 2009), while some others reported the reverse outcome (Olle, 2017; Olle et al. 2019).

1.4 Pea molecular markers diversity

Morphological markers for qualitative and quantitative traits of pea have long been developed and used for the selection of beneficial breeding materials. In recent years, significant progress has been made in molecular marker development, which greatly facilitated diversity analysis, genetic mapping of quantitative trait loci (QTL), and marker-assisted breeding (Pandey et al., 2021).

As a species belonging to family Fabaceae (Leguminosae), pea has a large genome size of about 4,5 Gb (Jain et al., 2014; Kreplak et al., 2019). According to Pandey et al. (2021), the large genome size of pea partly accounts for the delay in successful reference genome assembly and gene discovery in pea as opposed to other major food legumes (Kreplak et al., 2019). Despite this fact, the pea research community has come a long way in achieving a variety of genetic and omics resources, even before a reference genome was available (Figure 1.3.).

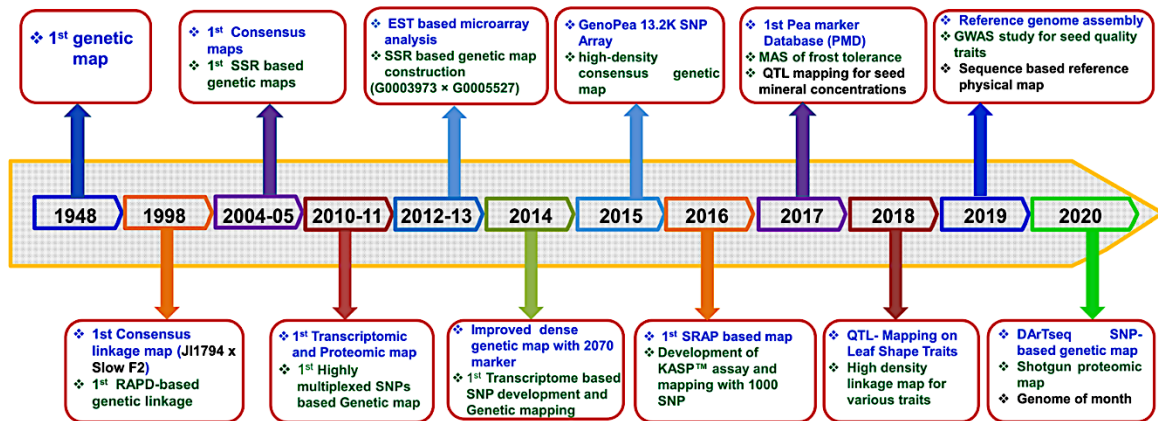


Figure 1.3 A road map representing the most important events in the evolution of Pea (*Pisum sativum* L.) genomics (Pandey et al., 2021).

Genetic diversity in a particular crop species is one of the most valuable assets for crop improvement. Along with the recent technical innovations, various kinds of molecular markers such as RAPD, ISSR, SSR, RBIP and SNPs were used to evaluate the genetic characterization of pea germplasm (Baranger et al., 2004; Tar'an et al., 2005; Nasiri et al., 2009; Jing et al., 2010; Sarikamış et al., 2010; Kwon et al., 2012; Jain et al., 2014; Ahmad et al., 2015; Tayeh et al., 2015). Nevertheless, the knowledge gap between researchers and breeders is till one of the major obstacles in many molecular breeding programs (Pandey et al., 2021).

1.5 Objectives of the thesis

The thesis contains three manuscripts (Chapters 2, 3 and 4) and is based on field experiments in 2017, 2018, and 2019 and on a study on genetic diversity by molecular markers.

The aim of the first manuscript is to compare the two leaf types (based on a diverse set of 24 normal-leafed and 30 semi-leafless cultivars from different breeding eras and backgrounds, including exotic genotypes) for general differences in yield and nitrogen content as well as the relationships between seed yield and straw yield and nitrogen content in seed and straw. Of special interest is the possibility to identify genotypes that combine a high protein content in the seed with a high nitrogen yield in the straw to increase the pre-crop value.

The target of the second manuscript is to evaluate the difference between two leaf types in seed and straw dry yield and other agronomic traits. In addition, the influence of plant length on nitrogen content and nitrogen yield of different leaf types is investigated. The material are near-isogenic bulks differing in leaf type and plant length composed of lines from crosses between normal-leafed and semi-leafless parents.

The goal of the third manuscript is the assessment of the genetic diversity of pea genotypes differing in leaf types (24 normal-leafed and 22 semi-leafless) using SNP markers, and to evaluate the contribution of leaf type to genetic distance.

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Chapter 2

Agronomic performance of normal-leaved and semi-leafless pea (*Pisum sativum* L.) genotypes

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BH conceptualized and administrated the project. HB and BH acquired funds and supervised. CT carried out the investigation, the data curation, and wrote the first draft. All authors reviewed and edited the manuscript.

Core ideas

1. A high protein yield of pea seeds can be combined with a high pre-crop value of the whole plant.
2. A positive correlation of seed and straw yield was observed in field pea.
3. A negative correlation of yield and nitrogen content was observed in field pea.
4. Normal-leaved peas were lower in seed and straw yield than semi-leafless peas,
5. but most of the tested normal-leaved genotypes were older and/or less adapted.

Abstract

Pea (*Pisum sativum* L.) is a major pulse crop important as feed and food. Due to the symbiosis with nitrogen-fixing bacteria, it is a valuable component of low-input cropping systems. The traditional cultivation of peas was limited by their high susceptibility to lodging. This problem was reduced, though not completely solved by a semi-leafless mutant. Almost all modern cultivars carry this mutant. It is still an open question, whether the lack of leaflets may have impaired the productivity. In organic farming, there is still interest in normal-leafed peas as they can better compete with weeds. To compare the two leaf types, 24 normal-leafed and 30 semi-leafless genotypes were evaluated in three environments. Semi-leafless genotypes had a higher seed (51%) and straw yield (40%) yield, but most normal-leafed genotypes were older or less adapted than the semi-leafless ones. Some newer normal-leafed cultivars achieved the same yield level, but their cultivation is only possible in mixtures with a supporting crop. Nitrogen content in seed (10%) and in straw (30%) was lower in semi-leafless genotypes. A negative correlation of yield and nitrogen content was observed in both leaf types. However, semi-leafless peas had a higher nitrogen yield in seed and in straw. No correlation between nitrogen yield in seed and straw was found, so it is possible to combine a high seed yield with a high nitrogen yield in the straw. This is particularly important in organic production systems, where peas are also grown to provide nitrogen to the following crop.

Keywords: Pea, leaf type, semi-leafless, normal-leafed, nitrogen content, nitrogen yield.

2.1 Introduction

Pea (*Pisum sativum* L.) is grown worldwide on about 10 million ha. About 2.8 million ha are used as green vegetables and more than 7 million ha as dry peas for animal feed and human nutrition. Peas are mainly cultivated in Europe, North America, China, and India. Globally, pea is the fourth most important legume crop after soy bean, groundnut, and common bean. In Europe, it is the most important cool season grain legume (FAOSTAT, 2019).

Pea seeds are rich in protein (Atta et al., 2004). Because of the symbiosis with atmospheric-nitrogen fixing rhizobacteria, peas do not require nitrogen fertilizer and sometimes they contribute nitrogen to the following crop (Burstin et al., 2018). Therefore, peas are of particular interest in organic crop rotations (Gollner et al., 2019), but they can also contribute to crop diversity and energy efficiency in conventional production (Nemecek et al., 2008).

Advances in breeding for increased yield led to higher harvest indices that are usually accompanied by high nitrogen harvest indices. Thus, the cultivation of these genotypes may be associated with a negative nitrogen balance (Gollner et al., 2019; Reiter et al., 2002). In order to achieve a positive nitrogen balance, it would be important to select for a high nitrogen yield of the straw that stays in the field.

For a long time, the main limitation for pea production in areas with precipitation close to the harvest season was the high susceptibility to lodging of the normal-leaved wild type and consequently severe problems with harvesting. Reductions in yield and seed quality have also been attributed to lodging. When peas are grown in a pure stand, they often exhibit severe lodging after flowering (Stelling, 1997), and large yield reductions of normal-leaved cultivars have been recorded in conventional management (Stelling, 1994). Contributing to this reduction is the large amount of foliage in leafed cultivars that shades lower plant parts and results in decreased light penetration and photosynthetic activity of the lower leaves, and the potential build-up of pathogens in the moist lower-canopy environment (Meadley & Milbourne, 1971). Among the many leaf mutants in pea, two are of practical importance: ‘afila’ (af) that replaces the leaflets with tendrils and ‘stipules reduced’ (st) that drastically reduces the size of the stipules (Mikic et al., 2011). Combining these two mutants (afaf stst) results in nearly leafless peas, and it was soon

observed that their yield is greatly reduced due to their small photosynthetic area (Hedley & Ambrose, 1981; Stelling, 1994). This was also observed in 'Filby,' the first fully leafless (afaf stst) UK pea cultivar, released in 1978 by Snoch (Mikic et al., 2011). Also the mutant stst alone resulted in significantly reduced yield. Therefore, semi-leafless genotypes are preferred, in which only the leaflets, but not the stipules, are modified (afaf StSt). Today semi-leafless pea cultivars account for more than 95% of the total dry pea production in western Canada, more than 80% in the European Union, and more than 30% in Russia (Mikic et al., 2011). All 23 spring pea cultivars on the recommended list in Germany are semi-leafless (Federal Plant Variety Office, 2020). They have also become dominant in Australia over the past decade because of their better resistance to lodging and hence easier harvesting (French, 2016). They allow more light and more air movement through the crop and consequently create less favorable conditions for fungal disease (Grevsen, 2003).

Normal-leafed cultivars are still of interest in organic agriculture due to their better competition against weeds (Gronle et al., 2014; Spies et al., 2011). They are also of interest as a genetic resource to increase protein content and to broaden the genetic diversity of the present breeding material. In addition, pea and cereal mixtures have been traditionally used in different parts of the world. Dry matter yields of the mixtures were generally higher than monoculture stands (Bedoussac et al., 2015; Rauber et al., 2001). In recent years, semi-leafless peas have become preferred in mixtures over the normal-leafed cultivars (Rauber et al., 2000, 2001). However, semi-leafless peas were reported to be less competitive than normal-leafed peas with greater leaf area, plant height, and shoot dry matter attributes (Semere & Froud-Williams, 2001). In addition, mixtures of normal-leafed and semi-leafless peas were reported to be successful (Syrový et al., 2015).

Comparisons of the performance of normal-leafed and semi-leafless pea genotypes can be found in the literature, but in most cases only a very limited number of genotypes have been included. The results are contradictory: When studying six field pea genotypes (two semi-leafless and four normal-leafed) in eight environments for dry matter and seed yield, Acikgoz et al. (2009) concluded that the semi-leafless cultivars newly registered at that time in Turkey had a significant 6% seed yield advantage over the normal-leafed genotypes. Studies by Cupic et al. (2013) illustrated that normal-leafed pea cultivars had a lower forage and dry matter yield but higher protein content, though these results were

not consistent over different seasons. Narits (2008) reported that semi-leafless cultivars had a higher seed yield (cited in Olle, 2017a), but this was not evident in the investigation of Olle (2017b). Kalev and Narits (2004, cited in Olle, 2017a) showed that in years when the weather conditions favored vegetative growth, normal-leafed types gave a higher yield and better quality than semi-leafless cultivars and vice versa. Normal-leafed cultivars also had a higher protein content than semi-leafless genotypes (Olle, 2017a). In another study of 13 pea cultivars, Olle et al. (2019) recorded that normal-leafed cultivars had the highest and most stable average yield. Only one out of four semi-leafless cultivars yielded a higher and more stable protein content. In Germany, a higher protein content in normal-leafed genotypes was observed both in winter pea (Quendt et al., 2014) and in spring pea (Lietzow et al., 2013). These studies with a limited number of genotypes did not cover the diversity present within the two leaf types.

Therefore, we analyzed a diverse set of 24 normal-leafed and 30 semi-leafless cultivars from different breeding eras and backgrounds, including exotic genotypes. One problem with this material, however, is that the leaf types differ in their genetic background, because many normal-leafed genotypes are older and less adapted than the more modern semi-leafless cultivars. This will be discussed.

The objectives of this study were to compare the two leaf types for general differences in yield and nitrogen content as well as the relationships between seed yield and straw yield and nitrogen content in seed and straw. Of special interest was the possibility to identify genotypes as a resource for combining a high protein content in the seed with a high nitrogen yield in the straw to increase the pre-crop value.

2.2 Materials and methods

2.2.1 Genotypes

The research material was collected from gene banks, seed companies, and breeders. Recently released to very old cultivars, as well as recent breeding lines and some genetic resources, were used. The characteristics of 24 normal-leafed and 30 semi-leafless genotypes are described in Table 2.1.

Table 2.1 Characteristics of 54 pea genotypes

Form s = spring, w = winter

Genotype	Form	Leaf type	Year of release	Use	Country of origin	Breeder/source	Reference
Alvesta	s	Semi-leafless	2008	Grain	Germany	KWS Lochow	[1],[2]
Astronaute	s	Semi-leafless	2013	Grain	Germany	Norddeutsche Pflanzenzucht	[1], [2]
Baccara	s	Semi-leafless	1992	Grain	France	S. A. Florimond Desprez	[2]
Camilla	s	Semi-leafless	2006	Grain	Austria	KWS Lochow	[3]
Casablanca	s	Semi-leafless	2007	Grain	Germany	KWS Lochow	[1]
Eiffel	s	Semi-leafless	1996	Grain	Denmark	Danisco Seed	[2]
Gambit	s	Semi-leafless	2011	Grain	Czechia	Selgen	[2]
James	w	Semi-leafless	2009	Grain	France	RAGT	[2]
KA 258 ^a	s	Semi-leafless	2016	Grain	Italy	CREA-FLC	
KA-L11 ^a	s	Semi-leafless	2016	Grain	Italy	CREA-FLC	
Cheyenne	w	Semi-leafless	1998	Grain	France	GAE Recherche	[3]
Kleopatra	s	Semi-leafless	2005	Grain	Germany	Südwestdeutsche Saatzeit	[3]
KWS La Mancha	s	Semi-leafless	2009	Grain	Germany	KWS Lochow	[1],[2]
Madonna	s	Semi-leafless	1999	Grain	Germany	Norddeutsche Pflanzenzucht	[1],[2]
Myster	w	Semi-leafless	2016	Grain	France	RAGT	[1],[2]
Navarro	s	Semi-leafless	2010	Grain	Germany	Norddeutsche Pflanzenzucht	[1]
Poseidon	s	Semi-leafless	2016	Grain	Czechia	Selgen	[2]
Radley	s	Semi-leafless	1989	Grain	UK	Booker Seeds	[3]
Respect	s	Semi-leafless	2006	Grain	Austria	Maribo Seed International	[3]
Rocket	s	Semi-leafless	2004	Grain	Germany	Erbengemeinschaft Dr. Hans Rolf Späth	[1]
Salamanca	s	Semi-leafless	2009	Grain	Germany	Norddeutsche Pflanzenzucht	[1],[2]
Santana	s	Semi-leafless	2000	Grain	Germany	KWS Lochow	[1],[2]
Solara	s	Semi-leafless	1984	Grain	Belgium	INNOSEEDS	[3]
Specter	w	Semi-leafless	2007	Grain	Austria	Werner Vogt-Kaute	[2]

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Tip	s	Semi-leafless	2013	Grain	Czechia	Selgen	[1],[2]
15834 ^a	s	Semi-leafless	2016	Grain	Switzerland	Getreidezüchtung Peter Kunz	
15850 ^a	s	Semi-leafless	2016	Grain	Switzerland	Getreidezüchtung Peter Kunz	
15852 ^a	s	Semi-leafless	2016	Grain	Switzerland	Getreidezüchtung Peter Kunz	
15853 ^a	s	Semi-leafless	2016	Grain	Switzerland	Getreidezüchtung Peter Kunz	
15859 ^a	s	Semi-leafless	2016	Grain	Switzerland	Getreidezüchtung Peter Kunz	
AF 447	s	Normal-leafed	<1935	Grain	Afghanistan	IPK Gatersleben	[4]
AF 448	s	Normal-leafed	<1935	Grain	Afghanistan	IPK Gatersleben	[4]
AF 467	s	Normal-leafed	<1935	Grain	Afghanistan	IPK Gatersleben	[4]
Akoja	s	Normal-leafed	2009	Grain	Germany	Norddeutsche Pflanzenzucht	[2]
Bohatyr	s	Normal-leafed	1980	Grain	Czechia	Selgen	[2]
Breslau	s	Normal-leafed	<1945	Grain	Germany	IPK Gatersleben	[4]
Cerosa	s	Normal-leafed	<1945	Grain	Germany	IPK Gatersleben	[4]
Dolores	s	Normal-leafed	2009	Green fodder	Germany	Norddeutsche Pflanzenzucht	[1],[2]
ET 118	s	Normal-leafed	<1948	Grain	Ethiopia	IPK Gatersleben	[4]
ET 336	s	Normal-leafed	<1949	Grain	Ethiopia	IPK Gatersleben	[4]
Florida	s	Normal-leafed	1993	Green fodder	Germany	Norddeutsche Pflanzenzucht	[1],[2]
GR 293 EW	w	Normal-leafed	1942	Grain	Greece	IPK Gatersleben	[4]
GR 409	w	Normal-leafed	1941	Grain	Greece	IPK Gatersleben	[4]
GR 440	s	Normal-leafed	1942	Grain	Greece	IPK Gatersleben	[4]
GR 5	s	Normal-leafed	1943	Grain	Greece	IPK Gatersleben	[4]
Grana	s	Normal-leafed	1997	Grain	Czechia	NORDSAAT	[2]
Grapis	s	Normal-leafed	1991	Grain	Poland	NORDSAAT	[2]
Klif	s	Normal-leafed	2008	Grain	Poland	Poznanska Hodowla Roslin	[2]
Natura	s	Normal-leafed	2007	Grain	Czechia	Selgen	[2]
Pandora	w	Normal-leafed	2014	Grain	Austria	Werner Vogt-Kaute	[2]
Pisum Vilmorin III	s	Normal-leafed	<1948	Grain	France	IPK Gatersleben	[4]
Protecta	s	Normal-leafed	2009	Grain	Czechia	Selgen	[2]
Rosakrone	s	Normal-leafed	1970	Green fodder	Germany	Kruse	[5]
RU 165	s	Normal-leafed	1945	Grain	Russia	IPK Gatersleben	[4]

^a Breeding line

[1] Federal Plant Variety Office (2017), [2] European Commission (2017), [3] CPVO (2021), [4] Genbankinformationssystem (GBIS) IPK Gatersleben (2017), [5] Federal Plant Variety Office (1997)

2.2.2 Field experiments

Seed lots of similar age were used to provide homogenous seed quality. To achieve this, most genotypes were multiplied in homogenous conditions before starting the field experiments. Generally, stand establishment was good for all genotypes. The field experiments were performed in a randomized complete block design with three environments in Goettingen, Germany (Reinshof in 2017, 2018, and Niedernjesa in 2018) and two replicates for each environment (Table 2.2). The plots were sown with a Hege-95 with 5 m² plot size, 100 seeds per m², and 6 rows per plot. The sowing times were on 21 Apr. 2017 as well as the 6 (Niedernjesa) and 18 (Reinshof) Apr. 2018. Plots were harvested end of July until early August. The field trial was harvested by hand in 2017 and by the HG-160 plot combine harvester in 2018.

Table 2.2 Experimental environments

	Reinshof 2017	Reinshof 2018	Niedernjesa 2018
Location	51°30'01.6"N, 9°55'50.4"E	51°30'01.6"N, 9°55'50.4"E	51°28'14.8"N, 9°55'34.1"E
Mean temperature April – July (°C) *	14.5	16.9	16.9
Precipitation (April – July (mm) *)	268	172	172
Pre-crop	Winter wheat	Winter wheat	Maize
Soil	Alluvial loess, silty loam	Alluvial loess, silty loam	Alluvial loess, silty loam
Altitude above sea level (m)	140	140	160

* Wetterstation Göttingen, Germany, www.wetterstation-goettingen.de

2.2.3 Data collection

Morphological and agronomic traits were assessed as follows: Light interception (LI) is the ratio of photosynthetically active radiation (PAR) measured above and below the canopy. Measurements were taken 20 cm above the canopy and 10–20 cm above the ground. Each plot was measured in three positions around noon (10:00 to 14:30) with an AccuPAR ceptometer LB80. Lodging (Lg) was scored about one week after flowering (2017) or three weeks after flowering (2018) as 1 (no lodging, plants upright) to 9 (plants flat on the ground). The plant length (PL) of each genotype was scored after flowering by measuring the length of five plants in different positions of the inner rows. Plot height (PlotH) was measured about two weeks before harvest from the ground level to the average height of each plot. Seed dry yield (SDY) was calculated from the amount of seed per plot multiplied by seed dry matter (%). To measure seed dry matter (%), a sample of about 150 g was dried at 60 °C for 4 days. To determine nitrogen content in seed (Nseed), these samples were milled to powder <0.2 mm by the RETSCH Ultra Centrifugal Mill ZM 200. Then, 15–16 mg of the milled sample was packed with aluminum paper as a small pill for C/N analysis by Vario EL cube with Advanced Purge and Trap Technology from Elementar. Nitrogen yield in seed (NYS) was computed by multiplying nitrogen content in the seed with seed dry yield. Straw dry yield (StrDY) was calculated from the amount of straw multiplied by straw dry matter (%). To measure straw dry matter (%) a sample of 100 g of shredded straw was dried at 60 °C for 48 hours. To determine nitrogen content in straw (Nstraw), these samples were cut into smaller pieces by a RETSCH Cutting Mill SM300 and subsequently milled to powder <0.2 mm by a RETSCH Ultra Centrifugal Mill ZM 200. Then, 16–18 mg of the milled sample was packed with aluminum paper as a small pill for C/N analysis by the Vario EL cube with Advanced Purge and Trap Technology from Elementar. Nitrogen yield in straw (NYStr) was computed by multiplying nitrogen content in straw and straw dry yield. Biomass yield (BY) is the sum of seed dry yield and straw dry yield. Total nitrogen yield (TNY) is the sum of nitrogen in seed and straw. The harvest index (HI) was estimated by dividing seed dry yield by biomass yield. The nitrogen harvest index (NHI) was calculated by dividing the nitrogen yield of seed by total nitrogen yield.

2.2.4 Statistical analysis

The analysis of variance (ANOVA), correlation coefficients, and estimates of heritability (h^2) were calculated using PLABSTAT software version 3A (Utz, 2011). The following ANOVA models were used:

Single environments: $Y_{ij} = \mu + g_i + r_j + gr_{ij}$

Multi-environments: $Y_{ijk} = \mu + g_i + e_k + r_{jk} + ge_{ik} + ger_{ijk}$

Including leaf type as a factor: $Y_{ijkl} = \mu + t_l + g_{il} + e_k + r_{jk} + te_{lk} + ge_{ilk} + tegr_{ijkl}$

in which Y_{ij} , Y_{ijk} , Y_{ijkl} are the observations of a plot, μ is the general mean, with the effects g_i for genotype i (g_{il} within leaf type), r_j for replicate j (r_{jk} within environment k), e_k for environment k , t_l for leaf type l , and with the respective interactions and the error terms gr_{ij} , ger_{ijk} and $tegr_{ijkl}$.

The analysis of variance for scored traits was first performed for all of 54 genotypes; then, we applied the same model for i) 24 normal-leafed genotypes and ii) 30 semi-leafless genotypes. To test the difference between the leaf types, a sum of squares for leaf types was calculated by subtracting the sum of squares of the normal-leafed group and the semi-leafless group from the sum of squares for all genotypes (Supplemental Table 2.7). Principal component analysis (PCA) and the figures were created by R using the packets *ggplot2*, *ggbiplot*, and *factoextra*. For the PCA, the data of the traits with different units were standardized by rescaling and centering the units.

2.3 Results

The analysis of variance for normal-leafed and semi-leafless peas (Table 2.3) shows significant differences for all traits between the two leaf types. The heritability was very high for all traits (>80%). The normal-leafed group intercepted 2.4 percentage points more light, while much more lodging was observed (scores of 7.9 and 2.4, respectively). The plants of the normal-leafed cultivars were about 19 cm longer than those of semi-leafless cultivars. Probably, most of the more recently released semi-dwarf cultivars also carry a mutation for short internode length (no information available). However, plant length is not significantly correlated with seed yield ($r = -0.27$ for semi-leafless and $r = -0.16$ for normal-leafed, see Tables 2.10 and 2.11). Nitrogen content in seed and straw was higher for normal-leafed genotypes in the range of approximately 10% and 30%, respectively). However, seed dry yield and straw dry yield of this group were lower than for the semi-leafless genotypes by 34% and 29%, respectively (The seed dry yield of all genotypes in three environments is given in Supplementary Table 2.8). In general, nitrogen yield in seeds is around twice as high as the nitrogen yield in straw for both leaf types (ratio 1.9 and 2.5, respectively). For both traits, a better performance was recorded for the group of semi-leafless cultivars, but the difference between the two groups is larger for nitrogen yield in the seeds, with 26.53 kg/ha in comparison to just 1.87 kg/ha in nitrogen yield in the straw. Consequently, harvest and nitrogen harvest indices were higher for semi-leafless genotypes.

Table 2.3 Heritability of morphological and agronomic traits and mean values of normal-leaved (n=24) and semi-leafless pea genotypes (n=30)

No.	Traits		Mean of all genotypes (n=54)	Mean of normal-leaved (n=24)	Mean of semi-leafless (n=30)	F _{test} normal vs. semi-leafless	Heritability (n=54)
1	Light interception (%)	LI	82.14	83.50	81.11	25.92**	0.85
2	Lodging	Lg	4.83	7.86	2.41	1890.16**	0.98
3	Plant length (cm)	PL	80.66	91.27	72.15	671.17**	0.98
4	Plot height (cm)	PlotH	61.74	55.58	66.80	221.24**	0.96
5	Seed dry yield (t/ha)	SDY	2.10	1.64	2.48	508.10**	0.96
6	Nitrogen content in seed (%)	Nseed	3.73	3.91	3.60	462.69**	0.96
7	Nitrogen yield in seed (kg/ha)	NYS	77.70	63.10	89.63	359.84**	0.95
8	Straw dry yield (t/ha)	StrDY	2.93	2.55	3.57	375.81**	0.95
9	Nitrogen content in straw (%)	Nstraw	1.15	1.30	1.03	309.06**	0.95
10	Nitrogen yield in straw (kg/ha)	NYStr	34.49	33.51	35.38	3.77+	0.88
11	Biomass yield	BY	5.04	4.20	5.72	653.28**	0.97
12	Total nitrogen yield	TNY	112.27	96.72	125.02	336.92**	0.94
13	Harvest index	HI	0.41	0.38	0.43	126.64**	0.94
14	Nitrogen harvest index	NHI	0.68	0.63	0.72	179.68**	0.94

+, *, ** = significant at p=0.1, p=0.05 and p=0.01, respectively

The results of the analysis of variance for seven important traits (seed and straw dry yield, nitrogen content in seed and straw, nitrogen yield in seed and straw, and total nitrogen yield) are shown in Table 2.4. The differences between normal-leaved and semi-leafless genotypes were highly significant for all traits, except nitrogen yield in straw, which was only significant at p = 0.10. The interactions with environments were highly significant for all traits, both for leaf types and for the variance within leaf types. Within each leaf type, there was a highly significant variation for all traits; it was always larger within the normal-leaved peas.

Table 2.4 Analysis of variance of seed and straw yield and nitrogen yield of 24 normal-leafed and 30 semi-leafless pea genotypes in three environments

Source of variation	Degrees of freedom	Mean square	Variance components	F _{test}
Seed dry yield				
Leaf type	1	55.0275	0.1700	508.10**
Normal-leafed	23	1.9811	0.3118	17.95**
Semi-leafless	29	1.3231	0.2039	13.28**
Leaf type x Environments	2	9.0527	0.0828	83.59**
Normal-leafed x Environments	44	0.2491	0.0694	2.26**
Semi-leafless x Environments	55	0.3361	0.1182	3.37**
Nitrogen content in seed				
Leaf type	1	8.3747	0.0258	462.69**
Normal-leafed	23	0.4860	0.0777	24.57**
Semi-leafless	29	0.1358	0.0200	8.56**
Leaf type x Environments	2	1.0018	0.0091	55.35**
Normal-leafed x Environments	44	0.0736	0.0269	3.72**
Semi-leafless x Environments	55	0.0498	0.0170	3.14**
Nitrogen yield in seed				
Leaf type	1	56050.0243	172.5131	359.84**
Normal-leafed	23	2292.0063	356.5511	15.01**
Semi-leafless	29	1607.0525	243.4120	10.96**
Leaf type x Environments	2	19055.1602	174.9944	122.33**
Normal-leafed x Environments	44	359.8682	103.5842	2.36**
Semi-leafless x Environments	55	411.6104	132.5149	2.81**
Straw dry yield				
Leaf type	1	38.0696	0.1171	375.81**
Normal-leafed	23	1.6925	0.2645	16.08**
Semi-leafless	29	1.4092	0.2185	14.38**
Leaf type x Environments	2	8.3693	0.0765	82.62**
Normal-leafed x Environments	45	0.3235	0.1091	3.07**
Semi-leafless x Environments	55	0.3627	0.1324	3.70**
Nitrogen content in straw				
Leaf type	1	6.0266	0.0185	309.06**
Normal-leafed	23	0.4094	0.0635	14.48**
Semi-leafless	29	0.1706	0.0265	14.54**
Leaf type x Environments	2	0.3177	0.0027	16.29**
Normal-leafed x Environments	45	0.0634	0.0175	2.24**
Semi-leafless x Environments	55	0.0195	0.0049	1.84**
Nitrogen yield in straw				
Leaf type	1	121.3196	0.2750	3.77+
Normal-leafed	23	312.8551	45.4710	7.82**
Semi-leafless	29	232.5595	34.7232	9.60**
Leaf type x Environments	2	1560.9964	14.1555	48.48**
Normal-leafed x Environments	45	117.1140	38.5424	2.93**
Semi-leafless x Environments	55	129.4798	52.6298	5.35**

Table 2.4 Continued				
Source of variation	Degrees of freedom	Mean square	Variance components	F_{test}
Total nitrogen yield				
Leaf type	1	63689.5076	63689.5076	336.92**
Normal-leafed	23	56890.6624	2473.5071	16.08**
Semi-leafless	29	48181.1387	1661.4186	8.47**
Leaf type x Environments	2	53500.8190	26750.4095	141.51**
Normal-leafed x Environments	44	21630.8018	491.6091	3.20**
Semi-leafless x Environments	55	31028.2119	564.1493	2.88**

+, *, ** = significant at p=0.1, p=0.05 and p=0.01, respectively

The differences between the two leaf types were generally consistent over the three environments, though considerable interactions were observed (Table 2.5). Seed dry yield of all genotypes in all environments is given in Table 2.8. The semi-leafless group showed a better performance in all environments in the traits seed dry yield, straw dry yield, nitrogen yield in seed, and total nitrogen yield. On the other hand, normal-leafed genotypes had higher nitrogen contents in seed and straw in each of the environments. When comparing the three environments, there was a much larger difference between the two leaf types in Reinshof 2017 compared to the two other environments for seed dry yield, straw dry yield, nitrogen yield in seed and total nitrogen yield. The most obvious interaction can be observed for seed dry yield and nitrogen yield in seed. At Reinshof 2017, semi-leafless peas had more than twice the yields of normal-leafed peas, whereas at the other two locations this difference was much smaller, though in the same direction. For nitrogen yield in straw, even a cross-over interaction occurs with higher values for semi-leafless peas at Reinshof 2017 and Reinshof 2018, but lower values at Niedernjesa 2018.

Table 2.5 Mean values for selected traits of normal-leafed (n=24) and semi-leafless (n=30) pea genotypes in three environments.

Traits	Reinshof 2017			Niedernjesa 2018			Reinshof 2018		
	Normal-leafed	Semi-leafless	F _{test}	Normal-leafed	Semi-leafless	F _{test}	Normal-leafed	Semi-leafless	F _{test}
Seed dry yield (t/ha)	1.06	2.56	458.77**	2.19	2.93	105.26**	1.60	1.88	14.49**
Nitrogen content in seed (%)	3.94	3.88	0.00	4.10	3.66	305.18**	3.68	3.26	295.13**
Nitrogen yield in seed (kg/ha)	41.37	99.74	412.96**	88.10	106.67	49.21**	57.90	60.72	0.00
Straw dry yield (t/ha)	3.00	4.23	174.64**	2.36	2.48	6.98*	2.32	2.97	160.04**
Nitrogen content in straw (%)	1.80	1.47	122.38**	1.15	0.81	170.23**	0.96	0.81	26.18**
Nitrogen yield in straw (kg/ha)	52.52	61.32	22.59**	26.67	20.24	59.04**	21.57	23.74	5.08*
Total nitrogen yield (kg/ha)	94.37	160.78	355.51**	114.77	126.94	16.88**	79.47	84.54	5.96*

** : significant at p=0.01

The relationship between yield and nitrogen content is of great interest. For all 54 genotypes, a highly significant negative correlation was observed between seed yield and nitrogen content in seed and between straw yield and nitrogen content in straw ($r = -0.69^{**}$ and $r = -0.58^{**}$, respectively) (Figures 2.1 and 2.2). Within the normal-leafed group, a significant negative relationship between yield and nitrogen content was recorded. For the semi-leafless group, the correlation between yield and nitrogen content was also negative, but not statistically significant. Despite this negative correlation, the semi-leafless types incorporated more nitrogen than normal-leafed cultivars due to their higher seed yield (see total nitrogen yield in Table 2.3).



Figure 2.1 Nitrogen content in seed and seed yield of 54 pea genotypes; mean of three environments

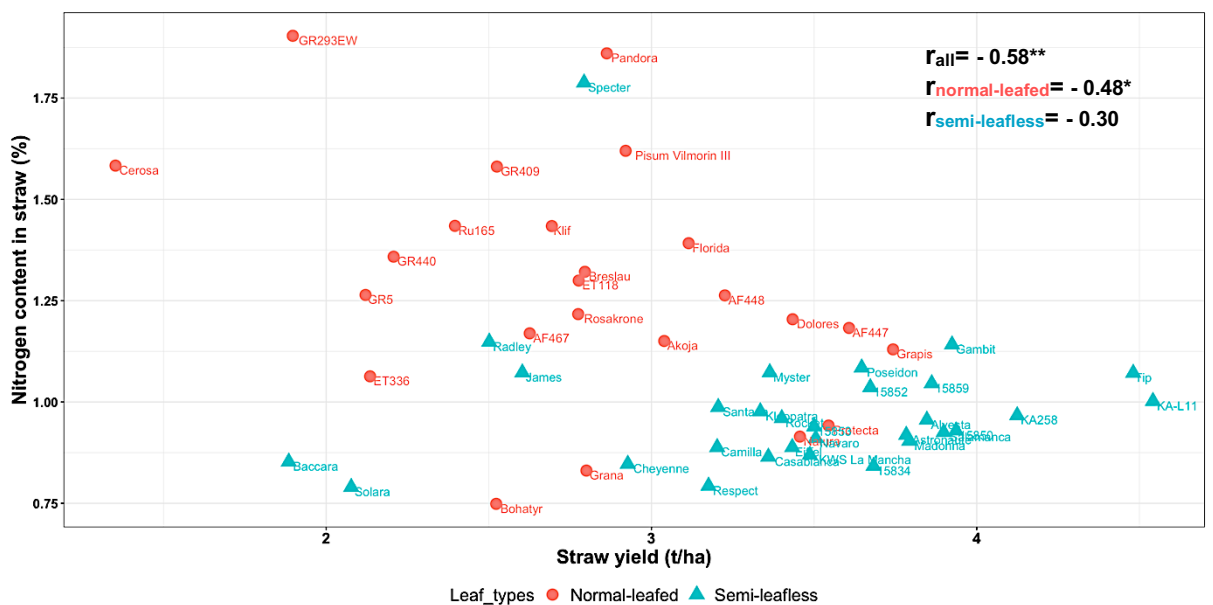


Figure 2.2 Nitrogen content in straw and straw yield of 54 pea genotypes, mean of three environments

Nitrogen yield in seed and nitrogen yield in straw were not correlated, neither for all genotypes nor within the two groups (Figure 2.3). Semi-leafless peas show a much higher nitrogen yield in seed, whereas the nitrogen yield in straw is similar for both leaf types. However, a few more recent normal-leaved cultivars have similar high nitrogen yield in seed as most of the semi-leafless cultivars.

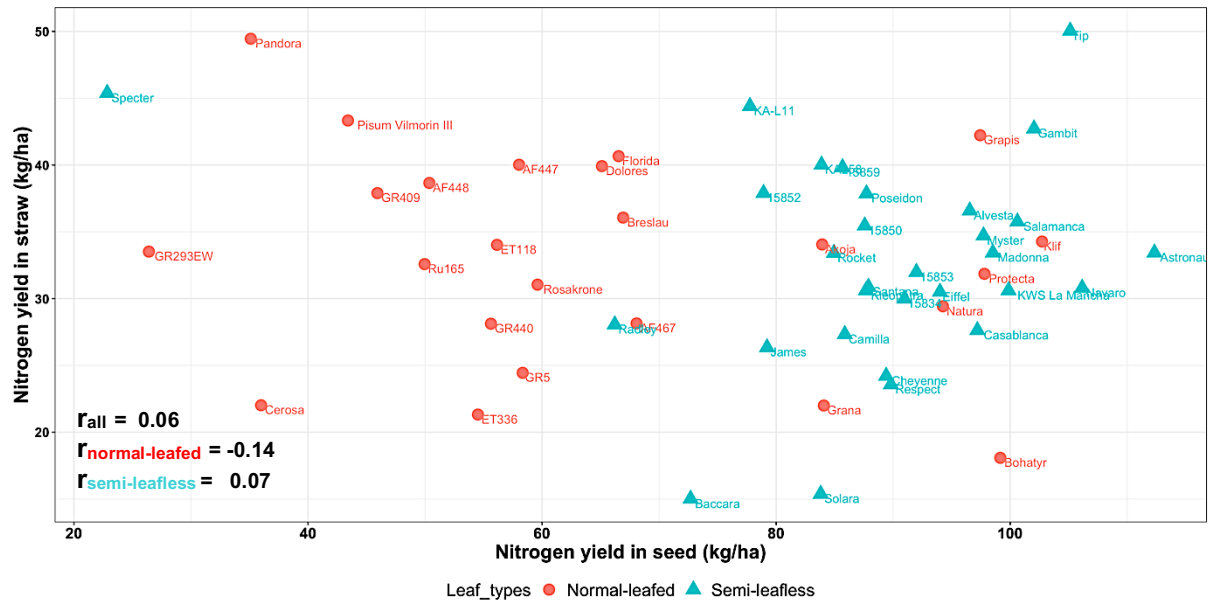


Figure 2. 3 Nitrogen yield in seed and straw of 54 pea genotypes; mean of three environments

The correlations between the seven most important traits for semi-leafless and normal-leaved genotypes are presented in Table 2.6. The correlations between all traits for all 54 genotypes and within both leaf types are given in Supplementary Tables 2.9 to 2.11. Generally, the correlations within the two leaf types are rather similar. Only the negative correlation of nitrogen content in seed with seed dry yield, nitrogen yield in seed, and total nitrogen yield is only significant for normal-leaved genotypes. Within the semi-leafless group, the nitrogen content in seed was not significantly correlated with other traits except with nitrogen content in straw ($r = 0.43^*$). Within the normal-leaved genotypes, nitrogen content in seed was positively correlated with nitrogen content in straw ($r = 0.67$), and negatively with seed dry yield, nitrogen yield in seed, and total nitrogen yield. A significant correlation of nitrogen yield in the seed with nitrogen content in straw was recorded for both leaf types.

Table 2.6 Correlation coefficients among selected traits of normal-leafed (n=24) and semi-leafless (n=30) pea genotypes

		Upper value: normal-leafed Lower value: semi-leafless					
Seed dry yield	(SDY)						
Nitrogen content in seed	(Nseed)	-0.67** -0.36					
Nitrogen yield in seed	(NYS)	0.98** 0.98**	-0.52** -0.17				
Straw dry yield	(StrDY)	0.60** 0.41*	-0.31 -0.06	0.61** 0.43*			
Nitrogen content in straw	(Nstraw)	-0.82** -0.87**	0.67** 0.43*	-0.76** -0.83**	-0.49* -0.28		
Nitrogen yield in straw	(NYStr)	-0.16 -0.20	0.28 0.30	-0.10 -0.14	0.57** 0.71**	0.42* 0.44*	
Total nitrogen yield	(TNY)	0.89** 0.90**	-0.41* -0.05	0.94** 0.93**	0.79** 0.69**	-0.59** -0.65**	0.26 0.23
		SDY	Nseed	NYS	StrDY	Nstraw	NYStr

Principal component (PC) analysis was applied to show the pattern of variation of all 54 genotypes and 14 traits. The first three PCs (PC1, PC2, PC3) explained almost 90% of the total variation, with the first component explaining 55.4%, the second component explaining 27.2%, and the third component explaining only 7.0% of the total variance (Supplementary Table 2.13). Thus, a biplot of PC1 and PC2 explained almost 83% of the total observed variance and is shown in Figure 2.4. The PC1 separated the two leaf types with some outlier genotypes like ‘Specter’ and ‘Radley’ of the semi-leafless group and ‘Bohatyr’, ‘Natura’, ‘Protecta’, ‘GR293EW’, and ‘Grapis’ of the normal-leafed group. PC1 was positively influenced by the traits SDY, NYS, TNY, BY, StrDY, PlotH, NHI, HI, and PL, with higher values for the semi-leafless group. The traits Nseed, Nstraw, and Lg had negative values of PC1 and were related to the normal-leafed group. On the other hand, the two groups of leaf types were not different in PC2. Some outlier genotypes were observed, in particular ‘Cerosa’, ‘Bohatyr’ (normal-leafed), and ‘Baccara’, and ‘Solara’ (semi-leafless). LI, NYStr, PL were traits with a positive influence on PC2. The eigenvalues for all PCs are given in Supplementary Table 2.13 and the principal components in Supplementary Table 2.12.

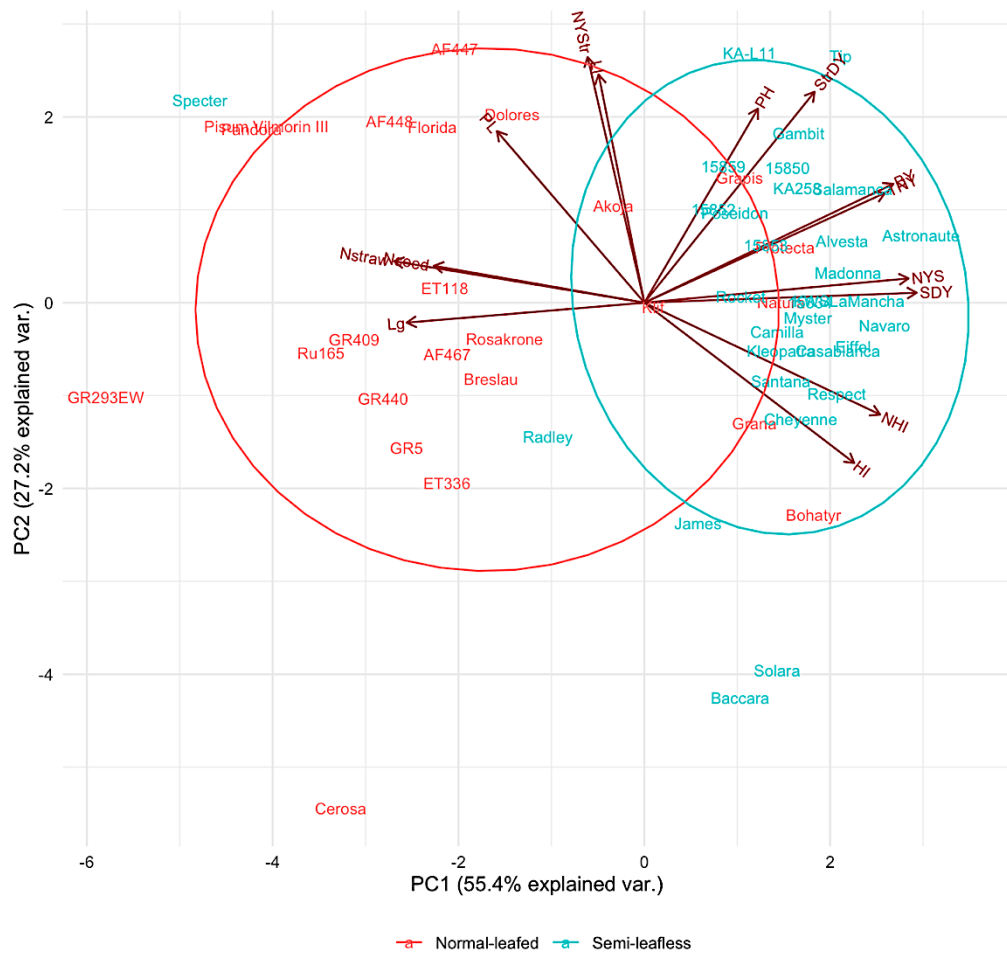


Figure 2.4 The first two principal components of a PCA of 54 pea genotypes and 14 traits.

Abbreviations of the traits: LI: light interception, Lg: Lodging, PL: Plant length, PlotH: Plot height, Nseed: Nitrogen content in seed, NYS: Nitrogen yield in seed, Nstraw: Nitrogen content in straw, NYStr: Nitrogen yield in straw, SDY: Seed dry yield, StrDY: Straw dry yield, BY: Biomass yield, TNY: Total nitrogen yield, HI: Harvest index, NHI: Nitrogen harvest index.

2.4 Discussion

Normal-leafed genotypes might be expected to produce higher seed and straw yields due to the larger area of photosynthesis. Stelling (1994) illustrated that normal-leafed peas had a higher yield potential than semi-leafless types in growing systems with or without supporting wire. Our results, however, do not indicate that semi-leafless types are generally associated with a loss in yield. Their average yield was significantly higher than the yield of normal-leafed genotypes. Additionally, the seed yield of normal-leafed genotypes is probably overestimated compared to practical cropping, where the yield of normal-leafed cultivars would be lower due to harvesting problems caused by lodging (Ambrose, 2008). In our experiment yield data were recorded from experimental plots, which were harvested very carefully.

The difference between normal-leaf and semi-leafless peas was examined through the evaluation of a diverse set of genotypes (30 semi-leafless and 24 normal-leafed) grown in different environments. Our results give insights into the capability of the two main plant architectures in pea. However, the genotypes of the two leaf types are genetically diverse and not directly comparable. This is also the case in the other published experiments and may explain the partially contradicting results in the literature. Most of the normal-leafed genotypes in our experiment were older and/or less adapted to the trial environments. Thus, experiments with genotypes segregating for leaf type within the same genetic background are required. The publication of results from such an experiment is in preparation.

The research material was clearly separated into two groups according to the leaf type (Figure 2.4), with a considerable overlap. Generally, the semi-leafless genotypes had a higher seed yield and straw yield (Table 2.3), but a lower nitrogen content. However, some normal-leafed cultivars, e.g., ‘Grapis’, ‘Protecta’, ‘Natura’, ‘Grana’, ‘Klif’, and ‘Dolores’ had the same yield level as semi-leafless cultivars (Figure 2.1). These cultivars were all released after the year 2000 (Table 2.1), supporting the assumption that the low average yield level of the normal-leafed genotypes is partly due to the fact, that many of these cultivars are relatively old.

The semi-leafless cultivar ‘Specter’ had the lowest seed yield, and other genotypes, as ‘Pandora’, ‘GR 409’, and ‘GR 293’, were also low-yielding (Figure 2.1). All of these are winter types. In the literature, winter peas have been reported to be more productive than spring peas (e.g. Chen et al. 2006; Urbatzka et al. 2011). In such experiments winter peas are sown in fall and spring peas in spring, resulting in a longer vegetation period for winter

peas. When winter peas are sown in spring, as in our experiment, they often start flowering late (data not shown).

The largest difference between the two leaf types is in lodging (Table 2.3); the normal-leafed group was seriously lodging right after flowering and during pod filling. Normal-leafed peas had on average a higher plant length than semi-leafless cultivars, also because many of the more recent semi-leafless cultivars carry the semi-dwarf mutant (Burstin et al. 2018). However, due to lodging, normal-leafed peas had a lower plot height. Although lodging is not a very serious problem for semi-leafless cultivars, a negative relationship between lodging and seed and straw yield was also observed in this group (Tables 2.9 to 2.11). Semi-leafless genotypes have also been reported lodging before harvest in other experiments (Schouls & Langelaan, 1994; Stelling, 1989; Uzun et al., 2005; Zajac et al., 2012). Tar'an et al. (2003) identified two major QTL for lodging resistance within semi-leafless material. Normal-leafed peas showed a more serious yield reduction due to lodging. This circumstance was also recorded by Singh and Srivastava (2015); serious lodging after flowering reduced irradiation to the leaflets on lower internodes of normal-leafed genotypes. Semi-leafless peas that stand more upright create better conditions for sunlight to penetrate to the lower part of the plants.

Some results were rather different between the three environments, in particular the seed yield and nitrogen yield of normal-leafed genotypes was very low in 2017 at Reinshof (Table 2.5) compared to 2018. In 2017, there was an unusually high rainfall in July before and during harvest (142 mm compared to 41 mm in 2018). This resulted in heavy lodging of the normal-leafed genotypes, but seed losses were low because the harvest was carried out by hand. Uzun et al. (2005) too observed that the differences between normal-leafed and semi-leafless peas were not consistent in different experimental years.

Within both kinds of leaf types, seed yield correlated positively with straw yield, and a similar relationship was observed for nitrogen content in seed and straw. A negative correlation between yield and nitrogen content was significant for seed and straw for all genotypes and within the normal-leafed group, but not within the semi-leafless group (Figures 2.1 and 2.2). A negative correlation between yield and protein content has frequently been observed in cereals (Simmonds, 1995). For pea, the situation is less obvious. In a meta-analysis of nine populations, Klein et al. (2020) observed only a slightly negative overall correlation (-0.11), which was inconsistent over populations. Despite the negative correlation between yield and nitrogen content in our experiment, yield was positively

correlated with nitrogen yield. Genotypes with higher yields therefore yielded more nitrogen per area. The negative correlation between yield and nitrogen content arises from the fact, that with increasing yield other components (mainly carbohydrates and fiber) increase more than nitrogen.

No correlation between nitrogen yield in seed and nitrogen yield in straw was observed within both leaf types as well as for all genotypes (Figure 2.3). This indicates that a high protein yield in the seed can be combined with a high amount of nitrogen in the straw, which is important for the pre-crop value of pea. The value of pea in crop rotations can be increased by choosing and breeding cultivars with high nitrogen yield in the seed plus high nitrogen yield in the straw.

Even some normal-leafed cultivars like 'Klif' fulfill these requirements. Due to the better competition against weeds, they are of special interest in organic production and other systems where the use of herbicides is not desirable or banned. However, due to their high susceptibility to lodging, normal-leafed peas will require mixed cropping with a supporting crop.

Supplementary Material

In the supplementary material additional information about the analysis of variance, detailed seed yield, the correlation of traits and the principal component analysis are given.

Table 2.7 Analysis of variance for seed dry yield

Source of variation	Degree of freedom	Sum of square	Mean square	Variance components	F _{test}	Degree of freedom - Numerator	Degree of freedom - Denominator
Genotypes	53	138.9614	2.6219	0.4189	24.21**	53	137
Leaf types	1	55.0275	55.0275	0.1700	508.10**	1	137
Normal-leafed	23	45.5644	1.9811	0.3118	17.95**	23	61
Semi-leafless	29	38.3695	1.3231	0.2039	13.28**	29	73
Environments	2	43.1055	21.5528	0.1986	198.99**	2	137
Replicates within E	3	2.3809	0.7936	0.0127	7.33**	3	137
Genotypes x E	101	47.551	0.4708	0.1812	4.35**	101	137
Leaf types x E	2	18.1055	9.0527	0.0828	83.59**	2	137
Normal-leafed x E	44	10.96	0.2491	0.0694	2.26**	44	61
Semi-leafless x E	55	18.4855	0.3361	0.1182	3.37**	55	73
Replicates x Genotypes x E	137	14.8388	0.1083	0.1083			
Total	296	246.8376					
Heritability	95.87						

E: Environments

**significant at p=0.01

Table 2.8 Mean values of seed dry yield of 54 pea genotypes in three environments

Genotype	Environment			Mean
	Reinshof 2017	Niedernjesa 2018	Reinshof 2018	
AF447	1.01	2.12	1.12	1.41
AF448	0.81	1.54	1.38	1.24
AF467	0.81	2.24	1.92	1.66
Akoja	1.30	2.76	1.94	2.00
Bohatyr	*	2.93	2.21	2.49
Bresslau	1.50	2.01	1.46	1.65
Cerosa	0.91	0.55	1.33	0.93
Dolores	1.13	2.78	1.47	1.79
ET118	0.63	2.40	1.52	1.52
ET336	0.66	2.20	1.53	1.46
Florida	1.26	1.91	1.48	1.55
GR293EW	0.18	0.92	0.69	0.59
GR409	0.84	1.71	1.23	1.26
GR440	0.81	1.73	1.52	1.35
GR5	0.93	1.85	1.60	1.46
Grana	2.11	2.98	2.08	2.39
Grapis	2.29	3.53	1.99	2.60
Klif	*	2.86	2.09	2.39
Natura	1.11	3.88	2.48	2.49
Pandora	0.55	1.20	0.92	0.89
Pisum Vilmorin III	0.88	1.15	0.96	1.00
Protecta	2.00	3.36	2.67	2.68
Rosakrone	1.08	2.28	1.59	1.65
Ru 165	0.53	1.77	1.18	1.16
15834	2.67	3.31	1.65	2.54
15850	2.47	3.05	1.91	2.48
15852	2.83	2.84	0.97	2.21
15853	3.46	2.47	1.72	2.55
15859	2.81	2.49	1.59	2.30
Alvesta	2.69	3.26	2.24	2.73
Astronaute	3.41	3.66	2.55	3.21
Baccara	*	2.23	2.22	2.14
Camilla	2.26	3.24	1.79	2.43
Casablanca	2.49	3.45	2.13	2.69
Eiffel	2.59	3.36	2.39	2.78
Gambit	2.76	3.67	1.98	2.80
James	3.15	1.96	1.40	2.17
KA258	2.82	2.95	1.67	2.48
KA-L11	1.78	3.09	1.79	2.22
Kayenne	2.81	2.56	2.48	2.62
Kleopatra	2.65	2.86	1.92	2.47
KWS La Mancha	2.99	3.00	2.10	2.69
Madonna	2.76	3.30	2.09	2.71
Myster	3.47	3.45	1.61	2.84

Navaro	3.02	3.76	2.15	2.98
Poseidon	2.62	2.76	1.46	2.28
Radley	1.51	1.69	1.88	1.69
Respect	*	3.02	2.26	2.56
Rocket	2.20	3.35	1.63	2.39
Salamanca	2.94	3.27	2.16	2.79
Santana	2.43	2.64	2.20	2.43
Solara	*	2.86	1.89	2.30
Specter	0.28	0.96	0.39	0.54
Tip	2.70	3.47	2.12	2.76
Mean	1.94	2.60	1.75	2.10

* missing value

Table 2.9 Correlation coefficients of 54 pea genotypes in three environments.

For abbreviation see Table 2.3

Lg	0.23													
PL	0.71**	0.59**												
PlotH	0.43**	-0.44**	0.29*											
SDY	-0.05	-0.81**	-0.46**	0.36**										
Nseed	0.24	0.69**	0.50**	-0.31*	-0.69**									
NYS	0.01	-0.77**	-0.40**	0.36**	0.98**	-0.56**								
StrDY	0.44**	-0.67**	0.03	0.80**	0.68**	-0.46**	0.67**							
Nstraw	0.08	0.73**	0.38**	-0.30*	-0.88**	0.72**	-0.83**	-0.57**						
NYStr	0.59**	-0.06	0.39**	0.62**	-0.06	0.15	-0.01	0.60**	0.28*					
BY	0.20	-0.81**	-0.24	0.63**	0.93**	-0.62**	0.92**	0.90**	-0.80**	0.28*				
TNY	0.18	-0.75**	-0.27*	0.52**	0.93**	-0.50**	0.96**	0.82**	-0.72**	0.27*	0.97**			
HI	-0.46**	-0.53**	-0.68**	-0.22	0.75**	-0.56**	0.73**	0.09	-0.74**	-0.57**	0.48**	0.54**		
NHI	-0.29*	-0.62**	-0.56**	-0.03	0.85**	-0.55**	0.84**	0.30*	-0.87**	-0.46**	0.64**	0.68**	0.95**	
	LI	Lg	PL	PlotH	SDY	Nseed	NYS	StrDY	Nstraw	NYStr	BY	TNY	HI	

Table 2.10 Correlation coefficients of 30 semi-leafless genotypes in three environments

For abbreviation see Table 2.3

Lg	0.13													
PL	0.62**	0.26												
PlotH	0.56**	0.03	0.93**											
SDY	-0.01	-0.72**	-0.27	-0.13										
Nseed	0.34	0.32	0.16	0.01	-0.36									
NYS	0.05	-0.71**	-0.24	-0.13	0.98**	-0.17								
StrDY	0.66**	-0.42*	0.61**	0.69**	0.41*	-0.06	0.43*							
Nstraw	0.16	0.76**	0.41*	0.21	-0.87**	0.43*	-0.83**	-0.28						
NYStr	0.69**	0.12	0.76**	0.67**	-0.20	0.30	-0.14	0.71**	0.44*					
BY	0.39*	-0.68**	0.21	0.34	0.84**	-0.24	0.84**	0.84**	-0.67**	0.31				
TNY	0.31	-0.66**	0.05	0.12	0.896**	-0.05	0.93**	0.69**	-0.65**	0.23	0.94**			
HI	-0.45*	-0.49**	-0.78**	-0.70**	0.75**	-0.31	0.72**	-0.23	-0.78**	-0.67**	0.30	0.45*		
NHI	-0.28	-0.64**	-0.64**	-0.52**	0.85**	-0.25	0.84**	0.02	-0.91**	-0.55**	0.51**	0.62**	0.94**	
	LI	Lg	PL	PlotH	SDY	Nseed	NYS	StrDY	Nstraw	NYStr	BY	TNY	HI	

Table 2.11 Correlation coefficients of 24 normal-leaved genotypes in three environments

For abbreviation see Table 2.3

Lg	-0.08																									
PL	0.76**	0.29																								
PlotH	0.61**	-0.41*	0.58**																							
SDY	0.23	-0.80**	-0.16	0.31																						
Nseed	0.01	0.60**	0.29	-0.17	-0.67**																					
NYS	0.28	-0.74**	-0.09	0.32	0.98**	-0.52**																				
StrDY	0.74**	-0.57**	0.44*	0.81**	0.60**	-0.31	0.61**																			
Nstraw	-0.20	0.61**	-0.01	-0.25	-0.82**	0.67**	-0.76**	-0.49*																		
NYStr	0.59**	0.06	0.46*	0.59**	-0.16	0.28	-0.10	0.57**	0.42*																	
BY	0.53**	-0.77**	0.14	0.62*	0.91**	-0.54**	0.91**	0.87**	-0.72**	0.21																
TNY	0.48*	-0.70**	0.08	0.52**	0.89**	-0.41*	0.94**	0.79**	-0.59**	0.26	0.96**															
HI	-0.39	-0.43*	-0.58**	-0.29	0.69**	-0.59**	0.65**	-0.09	-0.65**	-0.67**	0.36	0.39														
NHI	-0.17	-0.49*	-0.34	-0.09	0.80**	-0.56**	0.78**	0.12	-0.82**	-0.61**	0.53**	0.54**	0.95**													
	LI	Lg	PL	PlotH	SDY	Nseed	NYS	StrDY	Nstraw	NYStr	BY	TNY	HI													

Table 2.12 Principal components for fourteen traits

Traits	Coordinate													
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14
LI	-0,165	0,823	0,416	0,031	0,271	0,127	-0,174	-0,022	0,015	0,002	-0,004	0,001	0,000	0,000
Lg	-0,857	-0,072	0,390	0,110	0,089	-0,250	0,013	0,130	-0,089	-0,004	0,008	0,001	0,000	0,000
PL	-0,531	0,619	0,384	0,376	-0,081	-0,029	0,157	-0,096	0,064	-0,003	-0,006	-0,001	0,001	0,000
PlotH	0,410	0,700	-0,275	0,391	-0,272	-0,129	-0,146	0,026	-0,002	0,003	-0,002	-0,001	0,000	0,000
Nseed	-0,759	0,133	0,376	-0,311	-0,379	0,144	-0,030	0,039	-0,024	0,004	-0,013	-0,009	-0,003	0,000
NYS	0,953	0,088	0,229	-0,121	-0,064	-0,060	-0,010	-0,072	-0,044	0,001	0,031	-0,014	0,022	0,006
Nstraw	-0,902	0,150	-0,111	-0,307	-0,015	-0,191	-0,054	-0,107	0,021	-0,066	-0,013	0,012	0,003	-0,001
NYStr	-0,205	0,886	-0,175	-0,330	0,051	-0,121	0,042	0,066	0,088	0,031	0,020	-0,023	-0,004	0,002
SDY	0,982	0,036	0,123	-0,045	0,019	-0,066	0,008	-0,081	-0,060	-0,014	-0,004	-0,035	-0,015	-0,008
StrDY	0,614	0,761	-0,100	-0,047	0,034	0,087	0,083	0,112	-0,021	-0,045	-0,024	-0,001	0,013	-0,007
BY	0,898	0,429	0,023	-0,054	0,016	0,000	0,047	0,013	-0,040	-0,032	-0,020	0,011	-0,012	0,014
TNY	0,868	0,396	0,157	-0,222	-0,048	-0,086	0,026	-0,031	-0,020	0,049	0,003	0,042	-0,003	-0,006
HI	0,757	-0,579	0,241	-0,041	0,012	-0,135	-0,038	0,031	0,083	0,021	-0,058	-0,009	0,006	0,001
NHI	0,850	-0,404	0,296	0,022	-0,072	0,002	-0,026	0,074	0,099	-0,053	0,041	0,009	-0,006	-0,002

Abbreviations of the traits: LI: light interception, Lg: Lodging, PL: Plant length, PlotH: Plot height, Nseed: Nitrogen content in seed, NYS: Nitrogen yield in seed, Nstraw: Nitrogen content in straw, NYStr: Nitrogen yield in straw, SDY: Seed dry yield, StrDY: Straw dry yield, BY: Biomass yield, TNY: Total nitrogen yield, HI: Harvest index, NHI: Nitrogen harvest index.

Table 2.13 Eigenvalue and proportion of variance

Principal component	Eigenvalue	Variance explained (%)	Cumulative variance explained (%)
PC1	7,753	55,379	55,379
PC2	3,807	27,196	82,575
PC3	0,982	7,013	89,588
PC4	0,682	4,868	94,456
PC5	0,322	2,302	96,758
PC6	0,209	1,495	98,253
PC7	0,094	0,673	98,926
PC8	0,077	0,547	99,473
PC9	0,046	0,327	99,800
PC10	0,014	0,103	99,903
PC11	0,008	0,056	99,959
PC12	0,004	0,030	99,989
PC13	0,001	0,008	99,997
PC14	0,000	0,003	100,000

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Conflict of Interest Statement

The authors state there are no conflicts of interest

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Chapter 3

Trade-offs of major changes in the plant architecture - leaf type and plant length - of pea (*Pisum sativum* L.)

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Author contributions

BH conceptualized and administrated the project. HB and BH acquired funds and supervised. CT carried out the investigation, the data curation and wrote the first draft. All authors reviewed and edited the manuscript.

Abstract

In several crops, drastic changes in the phenotype e.g. the introduction of dwarfing genes in wheat and rice have been used to increase productivity and adaptation to agricultural production systems. In pea, the ‘*afila*’ mutation, which replaces the leaflets with tendrils has been combined with several mutants reducing plant length. Semi-leafless pea cultivars with reduced plant length are widely used in agricultural production due to their lodging resistance, whereas normal-leafed peas may have the potential for higher yield and better competition with weeds. Comparisons between semi-leafless and normal-leafed genotypes with comparable genetic backgrounds can hardly be found in the literature. Therefore, near-isogenic bulks were established to compare combinations of leaf types and plant lengths: semi-leafless, short; semi-leafless, long; normal-leafed, short; and normal-leafed, long. The bulks were established by mixing 10 recombinant inbred lines per bulk. This design was developed for four crosses. The bulks were evaluated in four environments for light interception, plant length, plot height, lodging, seed dry yield, straw dry yield, N content in seeds, N content in straw, N yield in seed, N yield in straw, biomass yield, total N yield, harvest index and N harvest index. Normal-leafed bulks yielded about 25% more seed dry matter but lodged severely, while semi-leafless bulks yielded almost 5% more straw dry matter. The normal-leafed type yielded about 26% more N in the seed but approximately 11% less N in straw compared to the semi-leafless type. The influence of plant height was small but significant for nearly all traits. Shorter genotypes lodged less, had a higher seed yield and harvest index, and a lower straw yield. In conclusion, normal-leafed peas had a higher yield potential than semi-leafless peas but lodged severely. They are of interest, especially for intercropping.

Key words: pea, *Pisum sativum*, leaf types, normal-leafed, semi-leafless, nitrogen yield.

3.1 Introduction

In several crops, drastic changes in the phenotype have been used to increase productivity and adaptation to agricultural production systems. Donald (1968) introduced the concept of “ideotype breeding” based on physiological models for the idealized appearance of a crop plant. The most well-known and most successful example for radical changes in plant architecture is the introduction of dwarfing genes in wheat and rice during the “Green Revolution” (Hedden 2003). In legumes, the advent of determinate genotypes in *Phaseolus vulgaris* has led to two distinctly different crops, i.e. the pole beans that are grown with support and bush beans. Recently, semi-leafless peas and determinate (“topless”) faba beans have mainly been investigated (Heath et al. 1994). In a current French pea project, several mutants for shoot and root architecture have been combined to define an optimal ideotype for production, even under stress conditions (Burstin et al. 2018).

Pea (*Pisum sativum* L.) is the most important grain legume in Europe. Peas are mainly used as protein-rich feed for animals as an alternative to soybeans and faba beans. There is a special interest in peas in organic agriculture because they could provide nitrogen (N) to the system via N fixation and produce grain rich in protein while improving soil N for succeeding crops (Carranca et al. 1999; Corre-Hellou and Crozat 2005). The value of pea as a preceding crop was also demonstrated by Babulicová (2016) because the grain and straw yield of winter wheat were significantly higher when grown after pea instead of winter barley.

For a long time, the main limitation for pea production has been the high susceptibility to lodging and, consequently, severe problems with harvesting (Stelling 1997) and seed quality. This problem has been largely reduced by using a mutant, which cause reduced

leaves. Many leaf mutants are known in pea, two of which are of practical importance: 'afila' (*af*), which replaces the leaflets with tendrils, and 'stipules reduced' (*st*), which drastically reduces the size of the stipules (Mikic et al. 2011). Combining these two mutants (*afaf stst*) results in nearly leafless peas, and their yield is largely reduced due to their small photosynthetic area (Hedley and Ambrose, 1981; Stelling, 1994). Therefore, semi-leafless genotypes are preferred, in which only the leaflets, but not the stipules, are modified (*afaf StSt*). Today, semi-leafless pea cultivars account for more than 95% of the total dry pea production in western Canada, more than 80% in the European Union, and more than 30% in Russia (Mikic et al., 2011). All 23 spring pea cultivars on the recommended list in Germany are semi-leafless (Federal Plant Variety Office, 2020).

However, effective weed management is a challenge, especially in organic farming. Therefore, normal-leafed cultivars are still of interest due to their better competition against weeds compared to semi-leafless cultivars (Spies et al., 2011; Gronle et al., 2014). Gollner et al. (2019) recommended normal-leafed peas for organic farming in dry regions because their higher shading ability increases competitiveness against weeds and reduces soil evaporation.

Experimental results on the yield difference between normal-leafed and semi-leafless cultivars are contradictory. According to Uzun and Acikgoz (1998), semi-leafless peas cultivars have advantages over normal-leafed peas in dry matter yield, seed yield and crude protein yield. Acikgoz et al. (2009) found that semi-leafless cultivars had around 6% higher seed yield and higher total dry matter yield than normal-leafed genotypes. Čupić et al. (2013) demonstrated that semi-leafless pea cultivars had higher forage yield and dry matter yield but lower in protein content, although the results were not consistent over environments. Singh and Srivastava (2015) found that the semi-leafless plant type had higher seed yield than the normal-leafed type and that the ideal plant type with high

tolerance to lodging was semi-leafless and with dwarf or semi-dwarf growth. The large amount of foliage that shades lower plant parts contributes to the lower yield of normal-leafed genotypes and results in decreased light penetration, lower photosynthetic activity of the lower leaves and the potential build-up of pathogens in the moist lower-canopy environment (Meadley and Milbourne, 1971; Grevsen 2003). In other studies, the advantage of normal-leafed genotypes over semi-leafless types was observed (Olle, 2017). Turk and Albayrak (2012) evaluated the forage yield and quality of different leaf types and concluded that the normal-leafed cultivar ‘Golyazi’ had the highest dry matter yield and crude protein yield, while the semi-leafless cultivar ‘Ulubatli’ had the lowest dry matter yield. Olle et al. (2019) reported that normal-leafed varieties had higher and more stable yield. In other studies, no clear difference between the two leaf types. Under dry conditions, both the normal-leafed and semi-leafless genotypes showed similar responses to variations in plant density and performance (Martin et al., 1994). The difference in grain yield, yield components and harvest index of semi-leafless and normal-leafed pea cultivars was not significant in some experiments (Mihailović et al., 2004; Mikić et al., 2006).

In the studies mentioned above, the cultivars of the two different leaf types originated from different breeding programmes and have different genetic backgrounds. This may explain the contradictory results. Therefore, we developed bulks of recombinant inbred lines from four crosses between a semi-leafless parent and a normal-leafed parent. Within the same leaf type, we differentiated between shorter and longer lines. The difference between such near-isogenic bulks from the same crosses should only be due to genes affecting leaf type and plant height. The objectives were to investigate the influence of leaf type and plant height on seed and straw dry matter yield, N content, and other agronomic traits in peas.

3.2 Materials and methods

3.2.1 Materials

The material comprised recombinant inbred lines in the F₆ generation of four crosses in which the parents differed in leaf type (Table 1). These crosses were performed in 2013 and 2014 and were numbered as crosses 1 (‘Grana’ x ‘Radley’), 7 (‘Klif’ x ‘Respect’), 10 (‘Wiato’ x ‘Rocket’), and 20 (‘Protecta’ x ‘Camilla’). In each cross, populations of 90 recombinant inbred lines were developed. Per cross, the 10 longest and 10 shortest normal-leafed lines and the 10 longest and 10 shortest semi-leafless lines were selected to assemble four near-isogenic bulks differing in leaf type and plant length: normal-leafed and long, semi-leafless and long, normal-leafed and short, and semi-leafless and short.

Table 3.1 Parental pea genotypes of four crosses ‘normal-leafed’ x ‘semi-leafless’

Crosses	Genotype	Form	Leaf types	Year of release	Use	Origin	Breeder/Source ^a	Reference
1	Grana	spring	Normal-leafed	1997	Grain	Czechia	NORDSAAT Saatzuchtgesellschaft mit beschränkter Haftung	Europäische Kommission (2017)
7	Klif	spring	Normal-leafed	2008	Grain	Poland	Poznanska Hodowla Roslin sp. z. o. o.	Europäische Kommission (2017)
10	Wiato	spring	Normal-leafed	1998	Grain	Poland	Poznanska Hodowla Roslin sp. z. o. o.	CPVO (2021)
20	Protecta	spring	Normal-leafed	2009	Grain	Czechia	Selgen	Europäische Kommission (2017)
1	Radley	spring	Semi-leafless	1989	Grain	UK	Booker Seeds Limited	CPVO (2021))
7	Respect	spring	Semi-leafless	2006	Grain	Austria	InterSaatzucht	Europäische Kommission (2017)
10	Rocket	spring	Semi-leafless	2004	Grain	German y	Erbengemeinschaft Dr. Hans Rolf Späth	Bundessortenamt (2017)
20	Camilla	spring	Semi-leafless	2006	Grain	Austria	Saatzucht Donau	CPVO (2021)

3.2.2 Methods

The field trials were conducted in four environments in Central Germany: Reinshof and Niedernjesa in 2018 and Reinshof and Deppoldshausen in 2019. Reinshof is situated at 51°30'01.6"N and 9°55'50.4"E at 140 m above sea level with a silty loam derived from alluvial loess. Niedernjesa is situated at 51°28'14.8"N and 9°55'34.1"E at 160 m above sea level with a silty loam derived from alluvial loess. Deppoldshausen is situated at 51°58'20.09"N and 9°96'86.71"E at 350 m above sea level with a loamy clay derived from limestone.

A split-plot design with two replicates was used. The main plots were the four bulks with the same combination of leaf type and plant length; sub-plot treatments were the four different crosses. Fifty seeds from each of the 10 lines per bulk were collected per plot. The plots were sown with a Hege-95 (*Hege*, Waldenburg, Germany) with a 5 m² plot size, 100 seeds per m², and 6 rows per plot. In 2018, the sowing times were 6 April in Niedernjesa and 18 April at Reinshof. In 2019, the sowing times were 24 April at Reinshof and 16 April in Deppoldshausen. A plot combine HG 160 was used for harvesting from 10 to 20 July 2018 and 30 July to 1 August 2019, when maturity was reached.

Morphological and agronomic traits were assessed as follows: **Light interception (LI)** was measured as the ratio of photosynthetically active radiation (PAR) measured above and below the canopy. Measurements were taken 20 cm above the canopy and 10–20 cm above the ground. Each plot was measured in three positions around noon (10:00–14:30) with an AccuPAR ceptometer LB80. Data were collected on 29 May 2018 and 17 and 24 June 2019. **Lodging (Lg)** was scored about three weeks after flowering, from 1 (no lodging, plants upright) to 9 (plants flat on the ground). **Plant length (PL)** was scored after flowering by measuring the length of 25 plants in different positions of the inner

rows. **Plot height (PlotH)** was measured about two weeks before harvest from the ground level to the average height of each plot. **Seed dry yield (SDY)** was calculated from the amount of seed per plot multiplied by the seed dry matter (%). To measure seed dry matter (%), a sample of about 150 g was dried at 60°C for 4 days. To determine the **N content in the seed (Nseed)**, these samples were milled to powder <0.2 mm by the RETSCH Ultra Centrifugal Mill ZM 200. Then, 15–16 mg of the milled sample was packed with aluminium paper as a small pill for C/N analysis by a Vario EL cube with Advanced Purge and Trap Technology from the Elementar Company. **N yield in seed (NYS)** was computed by multiplying the N content in the seed with the seed dry yield. **Straw dry yield (StrDY)** was calculated from the amount of straw multiplied by straw dry matter (%). To measure straw dry matter (%), a sample of 100 g of shredded straw was dried at 60°C for 48 hours. To determine the **N content in straw (Nstraw)**, these samples were cut into smaller pieces by a RETSCH Cutting Mill SM300 and subsequently milled to powder <0.2 mm by a RETSCH Ultra Centrifugal Mill ZM 200. Then, 16–18 mg of the milled sample was packed with aluminium paper as a small pill for C/N analysis by the Vario EL cube with Advanced Purge and Trap Technology from the Elementar company. **N yield in straw (NYStr)** was computed by multiplying the N content in straw and straw dry yield. **Biomass yield (BY)** was calculated as the sum of seed dry yield and straw dry yield. The **total N yield (TNY)** was calculated as the sum of N in seeds and straw. The **harvest index (HI)** was estimated by dividing the seed dry yield by the biomass yield. The **N harvest index (NHI)** was calculated by dividing the N yield of the seeds by the total N yield.

The analysis of variance (ANOVA), correlation coefficients, and estimates of heritability (h^2) were calculated using PLABSTAT software version 3A (Utz, 2011), after removing outliers in the data. The following ANOVA model was used:

$$Y_{ijklm} = \mu + e_i + r_{ji} + l_k + el_{ik} + p_l + pe_{li} + pl_{lk} + ple_{lki} + plre_{lkij} + c_m + ce_{mi} + cl_{mk} + cp_{ml} + cle_{mki} + cpe_{mli} + clp_{mkl} + clpre_{mklji}$$

in which Y_{ijklm} is the observations of a plot, μ is the general mean, with the effects e_i for environment i , r_{ji} for replicate j within environment i , l_k for leaf type k , p_l for plant length l , c_m for cross m , and with the respective interactions and the error terms $plre_{lkij}$ for main plots, and $clpre_{mklji}$ for sub-plots.

Principal component analysis (PCA) and the figures were created by the R programme with `ggplot2`, `ggbiplot` and `factoextra` packages. For PCA, the data of the traits with different units were standardized by rescaling and centring the units.

3.3 Results

The performance of the two leaf types showed highly significant differences for all 14 traits (Table 3.2) except N content in seed; the difference was not very large for some traits. The normal-leafed group intercepted almost 6% more light than the semi-leafless group, although measurements were taken late in the season. Lodging was much more severe for the normal-leafed type (7.15 vs. 2.67, respectively). The difference in plant length was only 0.8 cm, but due to lodging, the normal-leafed plots were about 5.2 cm shorter than the semi-leafless plots. Normal-leafed bulks yielded about 25% more dry yield, but semi-leafless bulks yielded almost 5% more straw dry matter. The difference of N content in seed and N content in straw between the two leaf types was very small but still significant. The normal-leafed type had a slightly higher N content in seeds but a lower N content in straw. The normal-leafed type yielded about 26% more N in seed but approximately 11% less N in straw compared to the semi-leafless type. The harvest index and the N harvest index were significantly higher for the normal-leafed type.

The average difference in plant length between the long and the short bulks was only about 5 cm. Therefore, the influence of plant length was small, though significant for most traits. Long plants showed significantly higher values than short plants in the traits of light interception, lodging, N content in seed, straw dry yield, N content in straw, and N yield in straw (Table 3.2) and significantly lower values for harvest index and N harvest index. The short plants were superior in seed dry yield and N yield in seed, but these differences showed low or no significance.

Table 3.2 The effect of leaf type (normal-leaved, semi-leafless) and plant length (long, short) on agronomic and morphological traits. Mean of near-isogenic bulks of recombinant inbred lines of four crosses.

Traits	Leaf types		F _{test} Leaf types	Plant length		F _{test} Plant length
	Normal-leaved	Semi-leafless		Long	Short	
Light interception (%)	84.24	78.38	106.69**	82.12	80.51	8.09**
Lodging	7.15	2.67	1037.74**	5.16	4.67	12.36**
Plant length (cm)	69.52	70.32	4.43*	72.50	67.34	186.97**
Plot high (cm)	65.66	70.86	213.37**	71.17	65.35	267.04**
Seed dry yield (t/ha)	2.16	1.72	102.88**	1.90	1.99	3.75+
Nitrogen content in seed (%)	3.42	3.40	3.77+	3.43	3.39	14.00**
Nitrogen yield in seed (kg/ha)	74.14	58.90	106.45**	65.50	67.53	1.90
Straw dry yield (t/ha)	2.70	2.83	26.59**	2.83	2.70	25.54**
Nitrogen content in straw (%)	1.08	1.17	83.19**	1.14	1.11	10.27**
Nitrogen yield in straw (kg/ha)	29.75	33.11	82.06**	32.83	30.03	56.76**
Biomass yield (t/ha)	4.95	4.65	19.86**	4.83	4.78	0.52
Total nitrogen yield (kg/ha)	103.88	92.01	48.58**	98.32	97.56	0.20
Harvest index	0.43	0.35	248.70**	0.38	0.40	15.50**
Nitrogen harvest index	0.70	0.61	238.59**	0.65	0.67	14.78**

** , * , + : significant at $p = 0.01$, 0.05 , and 0.1 , respectively

ANOVA was performed with the four main factors - environment, leaf type, plant length and crosses and the interaction between them. Table 3.3 shows the ANOVA for seed dry yield, in which environments and leaf types were highly significant at level $p = 0.01$, whereas the significance levels for cross and plant length were lower at $p = 0.05$ and $p = 0.10$, respectively. The effect of leaf type was not affected by plant length and cross, as

no significant interactions were observed). The interaction between leaf type and environment was significant, but the variance component for this interaction was much smaller than the variance component for leaf type, indicating that the effect of the leaf type on yield was rather consistent over the four environments investigated.

Table 3.3 Analysis of variance for seed dry yield

Source of variance	Degrees of freedom	Mean square	Variance components	F value
E	3	22.3305	0.6959	363.76**
R:E	4	0.3644	0.0189	5.94**
L	1	6.3158	0.0977	102.88**
EL	3	0.2565	0.0122	4.18*
P	1	0.2299	0.0026	3.75+
PE	3	0.0984	0.0023	1.60
PL	1	0.0737	0.0004	1.20
PEL	3	0.1054	0.0055	1.72
RPL:E	25	0.0614	-0.0058	0.73
C	3	0.3601	0.0086	4.26*
CE	9	0.3063	0.0277	3.62**
CL	3	0.0430	-0.0026	0.51
CP	3	0.0360	-0.0030	0.43
CEL	9	0.1444	0.0149	1.71
CPE	9	0.0755	-0.0023	0.89
CPL	3	0.1106	0.0032	1.31
CPEL:R	38	0.0846	0.0846	
Total	121			

E: Environment, L: Leaf type, R: Replication, P: Plant length, C: Cross
 **, *, +: significant at $p=0.01$, 0.05 , and 0.1 , respectively

In all four crosses, the seed dry yield of normal-leafed bulks was higher than for the semi-leafless bulks, both for short and long plants (Figure 3.1). Within the normal-leafed group, the seed dry yield of the short plants was higher than of the long plants for crosses 1, 7 and 20, but slightly lower for cross 10. Within the semi-leafless group, the short plants had a higher seed dry yield for all crosses except cross 1.

The difference in N content in seed between the two leaf types was generally small. Within the normal-leaved group, the N content in the seed of long plants was higher than that of short plants for crosses 1, 7, and 20, but not for cross 10. For the semi-leaf less group, higher N content was observed for long plants of crosses 7 and 10 but not for crosses 1 and 20.

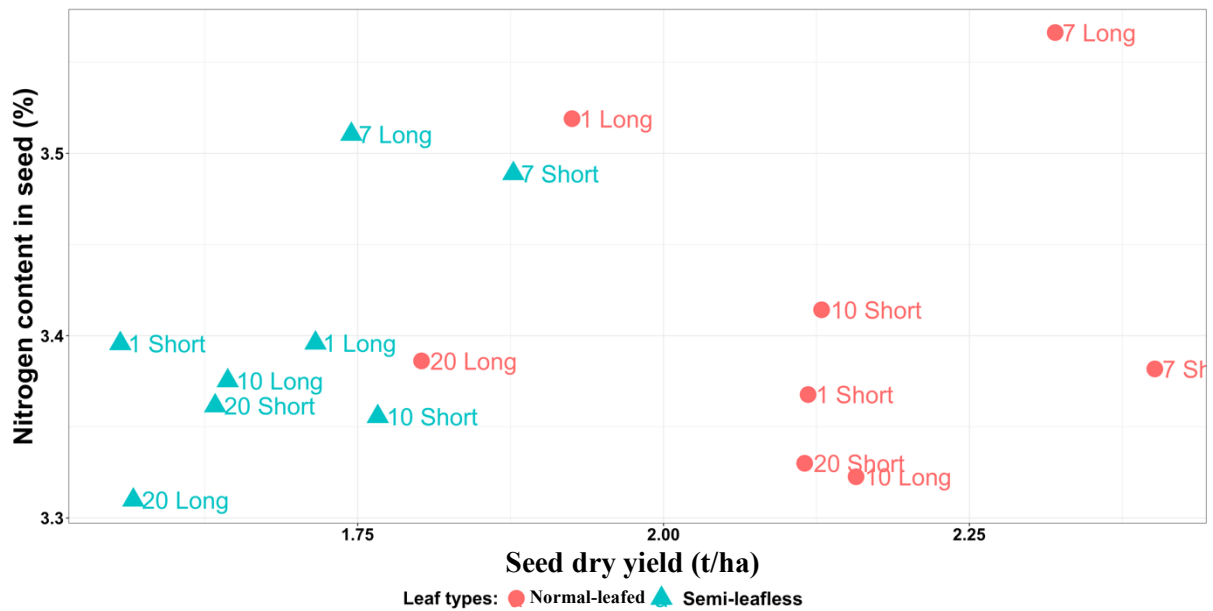


Figure 3.1 The effect of leaf type (normal-leaved, semi-leafless) and plant length (long, short) on seed dry yield and nitrogen content in seed of pea. Near-isogenic bulks of recombinant inbred lines of four crosses; mean of four environments.

The distribution of straw yield and nitrogen content in straw is shown in Figure 3.2. The general difference between the two leaf types in straw dry yield is not very obvious, whereas the nitrogen content in straw is clearly higher in the semi-leafless group. For the normal-leaved type, the nitrogen content in straw of long plants was higher than that of short plants in all crosses. However, in the semi-leafless group the influence of plant length on nitrogen content in straw is not clear, the longer plants have higher values in crosses 7 and 20 but lower values in crosses 1 and 10.

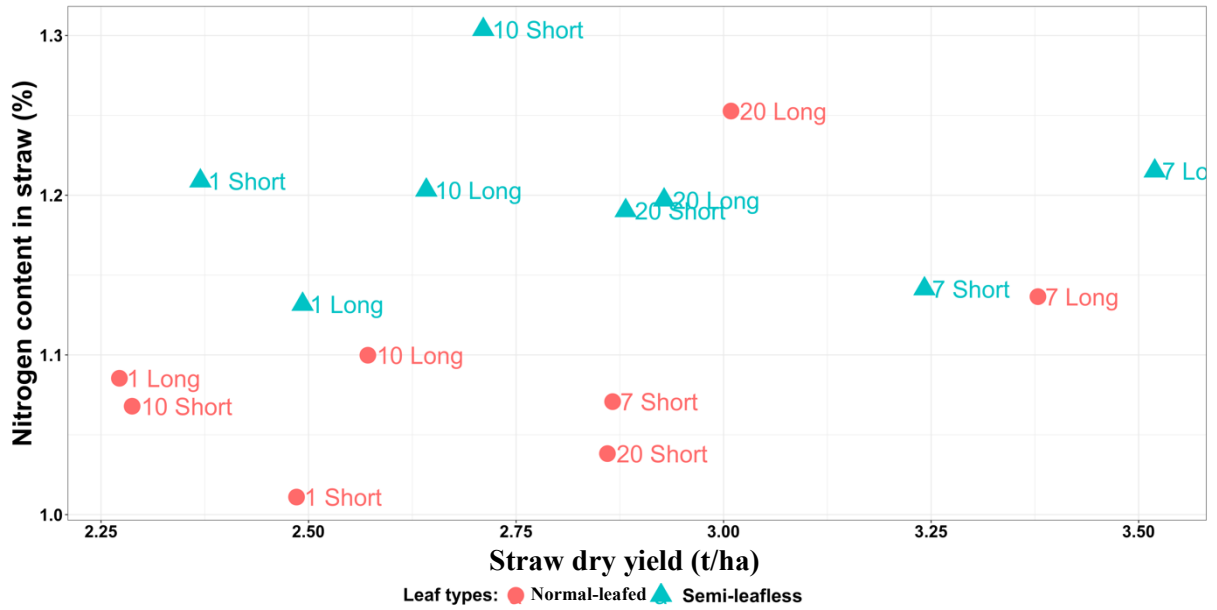


Figure 3.2 The effect of leaf type (normal-leaved, semi-leafless) and plant length (long, short) on straw dry yield and nitrogen content in the straw of pea. Near-isogenic bulks of recombinant inbred lines of four crosses; mean of four environments.

The seed dry yield of normal-leaved bulks was clearly higher than for the semi-leafless bulks for all crosses (Figure 3.3). All normal-leaved bulks were higher yielding than any of the semi-leafless bulks, except the long normal-leaved bulk of cross 20. Within the normal-leaved group, the seed dry yield of the short plants was higher than that of the long plants for the crosses 1, 7 and 20 but slightly lower for cross 10. Within the semi-leafless group, the short plants had a higher seed dry yield for all of crosses except cross 1. For straw dry yield, there is no clear general difference between the two leaf types. In most crosses, the long bulks had a slightly higher straw dry yield than the short bulks.

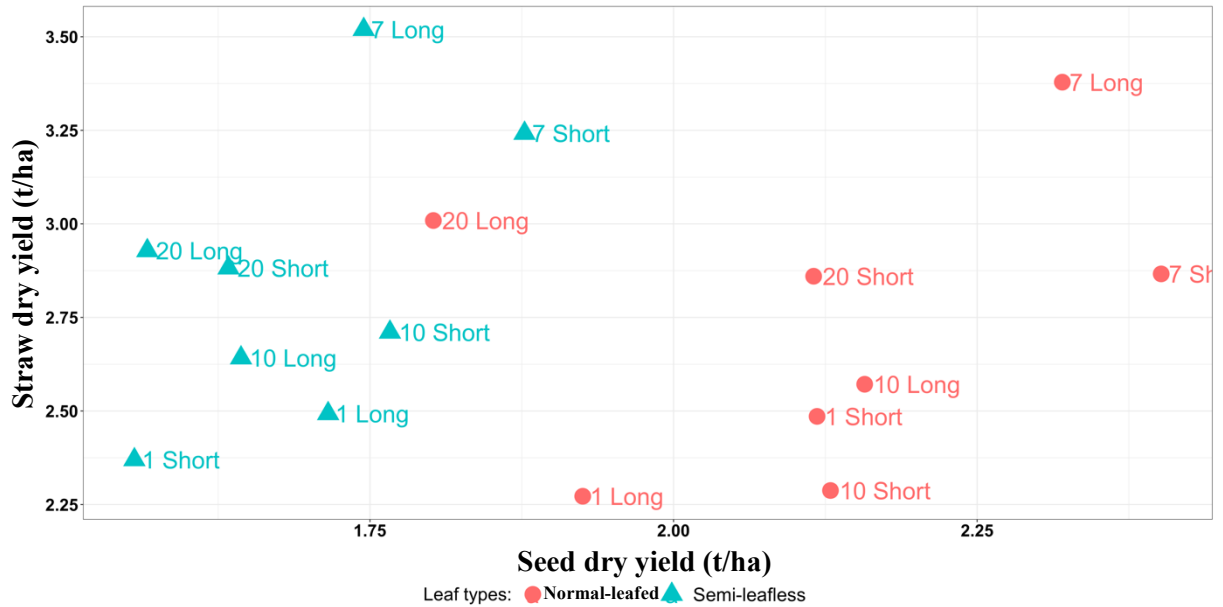


Figure 3.3 The effect of leaf type (normal-leafed and semi-leafless) and plant length (long, short) on seed dry yield and straw dry yield of pea. Near-isogenic bulks of recombinant inbred lines of four crosses; mean of four environments.

The difference in N content in seed between the two leaf types was generally small (Figure 3.4). Within the normal-leaved group, the N content in seeds of long plants was higher than that of short plants for crosses 1, 7, and 20 but not for cross 10. For the semi-leafless group, higher N content in seed was observed for long plants of crosses 7 and 10 but not for crosses 1 and 20. The N content in straw is clearly higher in the semi-leafless bulks. For the normal-leaved type, the N content in straw of long plants was higher than that of short plants in all crosses. In the semi-leafless group, the influence of plant length on N content in straw was not clear; the longer plants had higher values in crosses 7 and 20 but lower values in crosses 1 and 10.

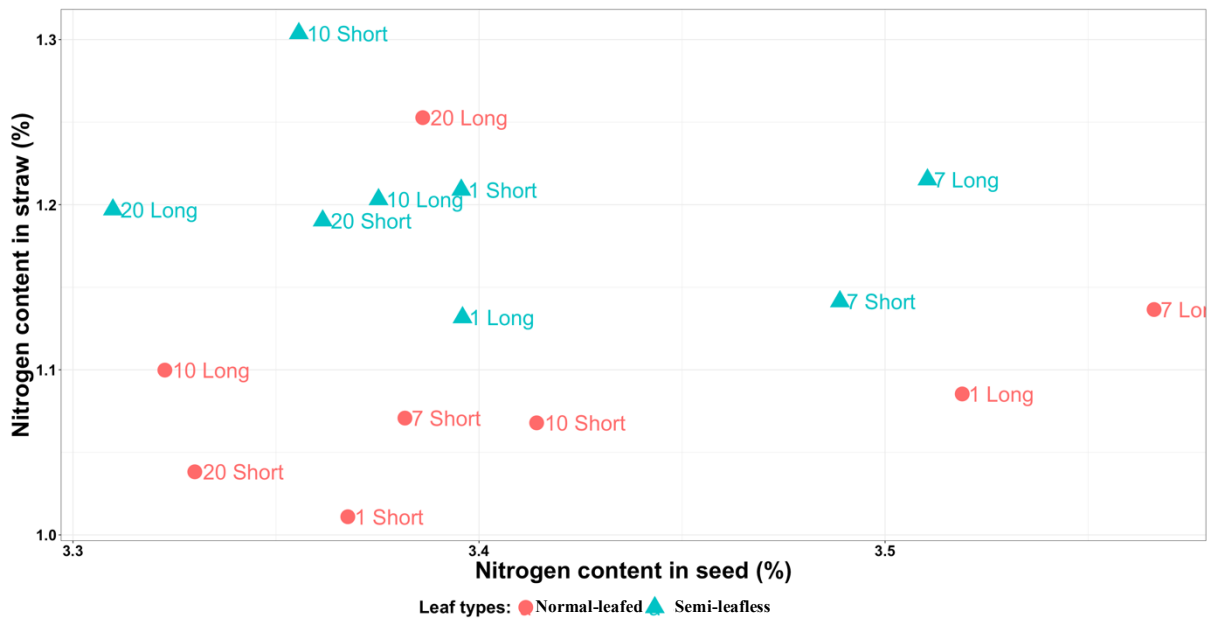


Figure 3.4 The effect of leaf type (normal-leaved, semi-leafless) and plant length (long, short) on nitrogen content in seed and nitrogen content in straw of pea. Near-isogenic bulks of recombinant inbred lines of four crosses; mean of four environments.

The N yield in seed and in straw is shown in Figure 3.5. The N yield in seed was clearly higher in normal-leaved bulks due their higher seed yield with similar N content. On the other hand, the N yield in straw was higher in the semi-leafless type because the straw yield was similar for both leaf types, but the N content in straw was higher in the semi-leafless type.

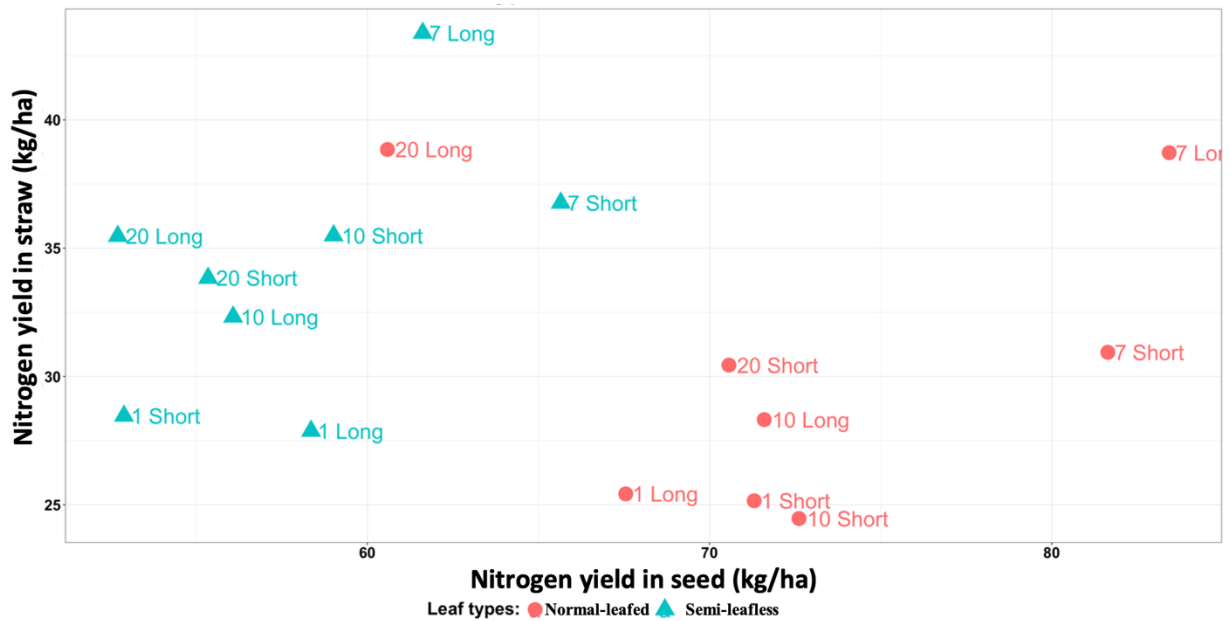


Figure 3.5 The effect of leaf type (normal-leaved, semi-leafless) and plant length (long, short) on nitrogen yield in seed and nitrogen yield in straw of pea. Near-isogenic bulks of recombinant inbred lines of four crosses; mean of four environments.

Figure 3.6 shows the first two principal components (PC1, PC2) of the principal component analysis, explaining about 85% of the total variation. The eigenvalues and proportion of variance are given in Table 3.4 and the principal component coefficients are given in Table 3.5 in the supplement. PC1 clearly separated the two leaf types, regardless of plant length and cross. The traits contributing to PC 1 were mainly the N content in straw, N yield in straw, lodging, seed dry yield, light interception, harvest index and N harvest index.

The PC2 described the difference between the crosses and the influence of plant length more than the difference between leaf types. Within both leaf types, the long plants had higher PC2 scores than the short plants, and all bulks from cross 7 have higher scores

than all bulks from cross 1. The traits mainly contributing to this difference were plant length, biomass yield, straw dry yield, N content in seed and total N yield.

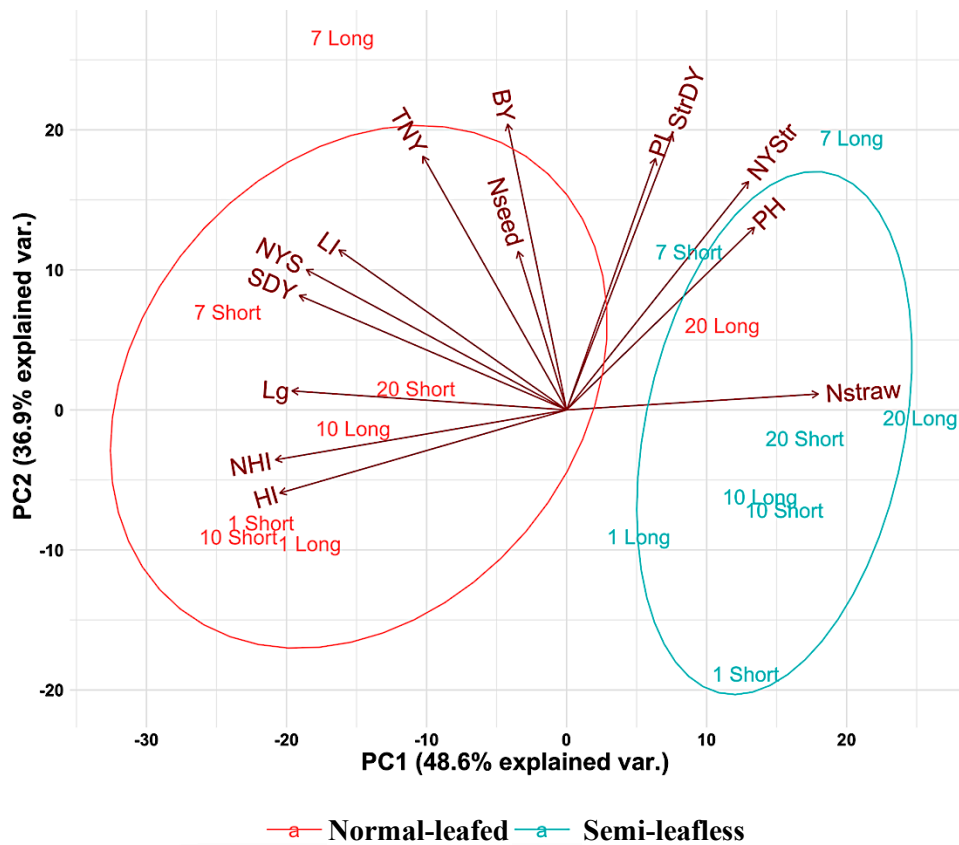


Figure 3.6 Principal component analysis of 14 agronomic and morphological traits of four near-isogenic bulks differing in leaf type and plant length for of four pea crosses.

Abbreviations of the traits: LI: light interception, Lg: Lodging, PL: Plant length, PH: Plot height, Nseed: Nitrogen content in seed, NYS: Nitrogen yield in seed, Nstraw: Nitrogen content in straw, NYSr: Nitrogen yield in straw, SDY: Seed dry yield, StrDY: Straw dry yield, BY: Biomass yield, TNY: Total nitrogen yield, HI: Harvest index, NHI: Nitrogen harvest index.

3.4 Discussion

To investigate the effect of the semi-leafless *af* mutant in pea, we compared near-isogenic bulks that differed in leaf type and plant length in four environments. The bulks were composed of recombinant inbred lines within four crosses of ‘semi-leafless x normal-leaved.’ Very few results on the effect of the *af* mutant in genetically comparable material have been published to date.

Lafond et al. (1981) compared two sets of near-isogenic lines – normal-leaved and semi-leafless – that were achieved from the sixth backcross to two recurrent parents. Genetic background greatly influenced the performance of semi-leafless genotypes. In the background of one parent, the normal-leaved genotype yielded similar results to the semi-leafless one, but it was markedly superior in the other parent’s background.

Goldman et al. (1992) compared a near-isogenic set of three foliage types. The normal-leaved type was slightly superior to the semi-leafless type (*af*) and much superior to the afila-tendriled acacia (*tac*) genotype. Stelling (1994) compared four sets of near-isogenic lines and found the highest yield potential in normal-leaved genotypes if supported by wire nettings to prevent lodging. Pesic et al. (2013) investigated the F₂ of crosses between a semi-leafless parent and 12 normal-leaved genotypes. The normal-leaved plants had a 10% higher yield, on average, in all crosses.

In agreement with these results, we observed a yield advantage for normal-leaved peas, which was even larger than in earlier experiments. Why are mainly semi-leafless cultivars grown today despite the yield advantage of normal-leaved peas? The obvious disadvantage of normal-leaved peas is their susceptibility to lodging, as shown in many previous studies (Elers, 2001; Olle, 2017; Gollner et al., 2019), in which normal-leaved

cultivars lodged severely after the flowering stage. Small experimental plots are carefully harvested to reduce losses during harvest. Under practical production in large fields, normal-leafed genotypes often have large losses during harvest due to lodging. Even in experimental plots, losses occur during the harvesting, threshing and processing of samples. We expect that the experimental error for seed yield is not affected by the leaf type. This may be different for straw yield and N content, as lighter parts, i.e. stipulae and leaflets, can easily break up into smaller fractions and lost more easily. The total amount of stipulae and leaflets appears to be larger in normal-leafed peas than in semi-leafless peas, although the latter typically have larger stipulae. Thus, the values for straw yield of normal-leafed bulks might be too low.

The difference between short and long bulks was only 5 cm on average. Nevertheless, plant length had statistically significant effects on nearly all traits. Shorter genotypes lodged less, had a higher seed yield and harvest index, and a lower straw yield. The effect of plant length would probably become more pronounced when using dwarf or semi-dwarf mutants, which have been known in pea for a very long time: dwarf peas were already used in crosses by John Goss in 1820 (Smýkal, 2014), and a dwarf gene, later called ‘Mendel's *le-1* mutation’ was used by Mendel (1866) to establish his laws of inheritance. Recently, dwarf and semi-dwarf genes have been exploited to create new cultivars with better standing ability (Burstin et al. 2018). However, such genotypes have not been successful thus far (GRDC, 2018; Rosewarne, n.d., Mcphee et al., 2017).

Due to the high sensitivity to lodging of normal-leafed peas to lodging, intercropping - predominantly with cereals - is the only cultivation method to avoid those disadvantages. In recent years, semi-leafless peas have been preferred in mixtures with cereals over normal-leafed cultivars (Rauber et al., 2001). Normal-leafed peas are more competitive

than semi-leafless peas in mixtures, probably due to the greater growth rate associated with a greater leaf area (Semere and Froud-Williams, 2001).

An interesting idea to grow normal-leafed and semi-leafless peas together in a mixture to combine the yield potential of normal-leafed peas with the superior standability of semi-leafless peas. Čupina et al. (2010) demonstrated that the intercropping of the two leaf types did not show significant differences in forage dry matter yield compared with normal-leafed pure stands. Mixtures between normal-leafed and semi-leafless peas were also analyzed by Syrový et al. (2015), showing that the combination of the two leaf types had a better ability to compete with weeds and a higher yield potential than monocultures and suggested an optimal combination of 25% normal-leafed and 75% semi-leafless peas, However, Carlsson et al. (2017) indicated that the benefits in terms of weed reduction and standing ability were not as strong in cultivars mixtures as in pea-wheat intercrops, which remain the most efficient mixtures. Recently, Gollner et al. (2019) observed no advantages in weed suppression and grain yield of pea leaf-type mixture compared to pea monocultures.

In conclusion, normal-leafed peas have a distinctly higher yield potential than semi-leafless peas. However, this yield potential cannot be used in monoculture production systems, mainly due to heavy lodging. Further research is required to optimize intercropping systems and to investigate the possibilities of improving standing ability of normal-leafed peas by introducing dwarf genes.

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Supplementary

Table 3.4 Eigenvalue and proportion of variance

Principal component	Eigenvalue	variance explained (%)	cumulative variance explained (%)
PC1	6,80	48.58	48.58
PC2	5,17	36.92	85.50
PC3	0,83	5.92	91.42
PC4	0,66	4.73	96.15
PC5	0,29	2.07	98.22
PC6	0,15	1.08	99.30
PC7	0,06	0.42	99.72
PC8	0,03	0.21	99.93
PC9	0,00	0.05	99.98
PC10	0,00	0.02	100.00
PC11	0,00	0.00	100.00
PC12	0,00	0.00	100.00
PC13	0,00	0.00	100.00
PC14	0,00	0.00	100.00

Table 3.5 The coordinates of 14 traits on the principal components

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14
LI	-0,760	0,534	-0,042	0,090	0,190	-0,290	-0,077	0,044	-0,001	0,000	0,000	0,000	0,000	0,000
Lg	-0,916	0,064	-0,056	0,099	0,315	0,183	-0,083	-0,058	0,004	-0,011	0,002	0,000	0,000	0,000
PL	0,296	0,839	0,264	0,302	0,120	0,139	0,047	0,108	0,005	0,004	0,000	0,000	0,000	0,000
PH	0,627	0,609	0,245	0,377	0,074	-0,101	0,090	-0,102	-0,003	0,000	0,000	0,000	0,000	0,000
SDY	-0,892	0,382	0,109	-0,204	-0,026	0,000	0,064	-0,009	0,001	0,008	0,012	0,007	0,002	0,000
Nseed	-0,162	0,529	-0,752	0,337	-0,118	0,027	0,023	0,002	0,002	0,004	0,003	0,001	0,000	0,000
NYS	-0,868	0,468	-0,005	-0,132	-0,045	-0,005	0,077	-0,002	0,028	-0,016	-0,005	-0,003	0,000	0,000
StrDY	0,353	0,919	0,055	-0,067	-0,127	0,024	-0,069	-0,013	-0,022	-0,013	0,003	-0,005	0,005	0,000
Nstraw	0,840	0,053	-0,301	-0,334	0,279	-0,022	0,101	0,016	-0,026	-0,010	0,002	-0,001	0,000	0,000
NYStr	0,607	0,762	-0,087	-0,179	0,065	0,043	-0,061	-0,025	-0,009	0,027	-0,008	0,004	0,001	0,000
BY	-0,197	0,954	0,106	-0,159	-0,118	0,016	-0,029	-0,003	-0,022	-0,001	0,005	-0,002	-0,006	0,000
TNY	-0,479	0,847	-0,051	-0,220	-0,007	0,018	0,039	-0,016	0,022	-0,001	-0,009	0,000	0,000	0,000
HI	-0,957	-0,278	0,020	0,002	0,045	0,015	0,056	-0,008	-0,016	0,033	0,001	-0,008	0,001	0,000
NHI	-0,971	-0,167	0,042	0,134	-0,059	0,021	0,036	0,007	-0,056	-0,012	-0,008	0,004	0,000	0,000

Abbreviations of the traits: LI: light interception, Lg: Lodging, PL: Plant length, PH: Plot height, Nseed: Nitrogen content in seed, NYS: Nitrogen yield in seed, Nstraw: Nitrogen content in straw, NYStr: Nitrogen yield in straw, SDY: Seed dry yield, StrDY: Straw dry yield, BY: Biomass yield, TNY: Total nitrogen yield, HI: Harvest index, NHI: Nitrogen harvest index.

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Chapter 4

Genetic diversity of pea (*Pisum sativum* L.) genotypes differing in leaf type using SNP markers^a

Submitted to Genetic Resources and Crop Evolution

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^a We are dedicating this paper to Wolfgang Ecke on the occasion of his retirement. For more than 25 years he supported countless students and scientists in our division in the analysis of molecular markers

Abstract

A collection of 46 pea (*Pisum sativum* L.) accessions, mostly from Europe, were analysed for genetic diversity using the GenoPea 13.2K SNP Array chip. Of these accessions were 24 normal-leaved and 22 semi-leafless. Principal components analysis (PCA) separated the peas into two groups characterized by the two different leaf types, although some genotypes were exceptions and appeared in the opposite group. Cluster analysis confirmed the two groups. A dendrogram showed larger genetic distances between genotypes in the normal-leaved group compared to semi-leafless genotypes. Both PCA and cluster analysis show that the two leaf types are genetically divergent. So normal-leaved peas are an interesting genetic resource, even if the target are semi-leafless varieties.

Key words: pea diversity, SNP, genetic distance, leaf types, normal-leaved, semi-leafless.

4.1 Introduction

Peas (*Pisum sativum* L.) first appeared in early Neolithic farming villages of the Near East (7000 to 6000 B.C.). They were closely associated with wheat and barley production (Zohary & Hopf, 1973). Peas were then quickly distributed across Southwest Asia, the Mediterranean basin and temperate Europe, Ethiopia, and northwestern India (Weiss & Zohary, 2011). Peas are adapted to a wide range of climates and altitudes and, as a result, the diversity of the pea germplasm is very large (Burstin et al., 2015).

Today, peas are grown in many countries across Europe, North America, and Asia (Smýkal et al., 2012). Nevertheless, as for many other crops, in present plant breeding only a small part of the diversity is used. Especially, genetic diversity available in wild *Pisum* species has been poorly exploited (Jing et al., 2010; Ellis, 2011; Smýkal et al., 2011).

Pea is a diploid ($2n=2x=14$) species of the *Fabaceae* and has a genome size of about 4,500 Mb (Jain et al., 2014). Since the first genetic map which was published in 1948 (Lamprecht, 1948), knowledge of the pea genome has expanded consistently along with the development of molecular markers including RAPDs, SSRs, SRAPs and SNPs (Pandey et al., 2021). SNP markers are abundant and amenable to high-throughput genotyping (Tayeh et al. 2015b; Jha et al. 2017; Desgroux et al. 2018; Gali et al. 2018; Aznar-Fernández et al. 2020).

Thanks to the development of molecular markers in recent decades, various genetic diversity studies have been performed in pea. In an early study of Baranger et al. (2004), 121 protein-based and PCR-based markers (RAPD, ISSR, STS, SSR, Isozymes, Storage proteins) were used to investigate the genetic relationships and assess the genetic diversity of 148 pea accessions mostly from Europe, and from China and USA. Three types of molecular markers RAPD, ISSR, SSR were used to estimate the genetic relation among 65 pea varieties (Tar'an et al. 2005). Smýkal et al. (2008) screened materials originating from Europe, America and Asia by using SSR and RBIP markers. Zong et al. (2009) used 21 informative microsatellite loci to assess and compare the genetic diversity among pea genotypes from within and outside China. Kwon et al. (2012) analysed genetic diversity and population structure of 285 accessions in a pea collection of Western Regional Plant Introduction Station, United States Department of Agriculture (USDA) by using RAPD, SCAR, and SSR markers. Burstin et al. (2015) concluded that despite the limited number of SNP markers used in their study, these markers proved to be most efficient in describing the genetic structure of the pea collection compared to SSR and RBIP markers. Benefiting from next-generation sequencing

technology, a large number of gene-based SNP markers have been developed for pea genetic improvement. The first SNP array, GenoPea 13.2 K SNP, was developed from 12,802 transcript-derived SNPs following the re-sequencing of 16 diverse pea accessions (Tayeh et al. 2015a).

In pea there are four basic leaf types: the wild type or normal-leafed with leaflets and tendrils, tendril-less (homozygous recessive mutation for the *Tendriless* gene), afila or semi-leafless without leaflets (homozygous recessive mutation of the *Afila* gene), and afila-tendril-less (the combination of both mutations) (Mikić et al. 2011). Today, semi-leafless cultivars are mainly used in the main production areas of pea such as western Canada, European Union, Russia, Australia (Mikić et al. 2011; French 2016), as this leaf type shows very much reduced lodging and can easier be harvested. However, the development of new cultivars by exploiting only semi-leafless peas could narrow down the genetic diversity.

The production of pea is facing new challenges such as resistance to biotic and abiotic stresses and higher seed quality. Therefore, it is necessary to exploit a wide range of genetic resources including normal-leafed accessions. A comprehensive comparison of the two leaf types with a larger number of accessions using SNPs markers has not yet been carried out to our knowledge. The main aim of this study was to assess the genetic diversity of 46 pea cultivars of different leaf type, and to evaluate if there is a relationship between leaf type and genetic distance.

4.2 Materials and Methods

4.2.1 Plant materials

Germplasm consisted of 46 pea genotypes from various gene banks, seed companies and research institutes released from before 1935 to 2016. It consists of 24 normal-leafed and 22 semi-leafless accessions. The majority are European spring cultivars for grain usage, but some winter types, green fodder cultivars and exotic accessions from Asia and Africa are included (Table 4.1). Information on agronomic traits can be found in Tran et al. (2022).

Table 4.1 Characteristics of 46 pea genotypes

s = Spring, w = Winter

Genotype	Form	Leaf type	Year of release	Use	Country of origin	Breeder/source ^c	Reference
Alvesta	s	Semi-leafless	2008	Grain	Germany	KWS Lochow	[1], [2]
Astronaute	s	Semi-leafless	2013	Grain	Germany	Norddeutsche Pflanzenzucht	[1], [2]
Camilla	s	Semi-leafless	2006	Grain	Austria	KWS Lochow	[3]
Casablanca	s	Semi-leafless	2007	Grain	Germany	KWS Lochow	[1]
Eiffel	s	Semi-leafless	1996	Grain	Denmark	Danisco Seed	[2]
Gambit	s	Semi-leafless	2011	Grain	Czechia	Selgen	[2]
James	w	Semi-leafless	2009	Grain	France	RAGT	[2]
KA 258 ^a	s	Semi-leafless	2016	Grain	Italy	CREA-FLC	[4]
KA-L11 ^a	s	Semi-leafless	2016	Grain	Italy	CREA-FLC	[4]
Cheyenne	w	Semi-leafless	1998	Grain	France	GAE Recherche	[3]
Kleopatra	s	Semi-leafless	2005	Grain	Germany	Südwestdeutsche Saatzeit	[3]
Madonna	s	Semi-leafless	1999	Grain	Germany	Norddeutsche Pflanzenzucht	[1], [2]
Myster	w	Semi-leafless	2016	Grain	France	RAGT	[1], [2]
Navarro	s	Semi-leafless	2010	Grain	Germany	Norddeutsche Pflanzenzucht	[1]
Poseidon	s	Semi-leafless	2016	Grain	Czechia	Selgen	[2]
Radley	s	Semi-leafless	1989	Grain	UK	Booker Seeds	[3]
Respect	s	Semi-leafless	2006	Grain	Austria	Maribo Seed International	[3]
Rocket	s	Semi-leafless	2004	Grain	Germany	Erbengemeinschaft Dr. Hans Rolf Späth	[1]
Salamanca	s	Semi-leafless	2009	Grain	Germany	Norddeutsche Pflanzenzucht	[1], [2]
Santana	s	Semi-leafless	2000	Grain	Germany	KWS Lochow	[1], [2]

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Specter	w	Semi-leafless	2007	Grain	Austria	Werner Vogt-Kaute	[2]
Tip	s	Semi-leafless	2013	Grain	Czechia	Selgen	[1], [2]
AF 447	s	Normal-leafed	<1935	Grain	Afghanistan	IPK Gatersleben	[5]
AF 448	s	normal-leafed	<1935	Grain	Afghanistan	IPK Gatersleben	[5]
AF 467	s	normal-leafed	<1935	Grain	Afghanistan	IPK Gatersleben	[5]
Akoja	s	normal-leafed	2009	Grain	Germany	Norddeutsche Pflanzenzucht	[2]
Bohatyr	s	normal-leafed	1980	Grain	Czechia	Selgen	[2]
Breslau	s	normal-leafed	<1945	Grain	Germany	IPK Gatersleben	[5]
Cerosa	s	normal-leafed	<1945	Grain	Germany	IPK Gatersleben	[5]
Dolores	s	normal-leafed	2009	Green fodder	Germany	Norddeutsche Pflanzenzucht	[1], [2]
ET 118	s	normal-leafed	<1948	Grain	Ethiopia	IPK Gatersleben	[5]
ET 336	s	normal-leafed	<1949	Grain	Ethiopia	IPK Gatersleben	[5]
Florida	s	normal-leafed	1993	Green fodder	Germany	Norddeutsche Pflanzenzucht	[1], [2]
GR 293 EW	w	normal-leafed	1942	Grain	Greece	IPK Gatersleben	[5]
GR 409	w	normal-leafed	1941	Grain	Greece	IPK Gatersleben	[5]
GR 440	s	normal-leafed	1942	Grain	Greece	IPK Gatersleben	[5]
GR 5	s	normal-leafed	1943	Grain	Greece	IPK Gatersleben	[5]
Grana	s	normal-leafed	1997	Grain	Czechia	NORDSAAT	[2]
Grapis	s	normal-leafed	1991	Grain	Poland	NORDSAAT	[2]
Klif	s	normal-leafed	2008	Grain	Poland	Poznanska Hodowla Roslin	[2]
Natura	s	normal-leafed	2007	Grain	Czechia	Selgen	[2]
Pandora	w	normal-leafed	2014	Grain	Austria	Werner Vogt-Kaute	[2]
Pisum Vilmorin III	s	normal-leafed	<1948	Grain	France	IPK Gatersleben	[5]
Protecta	s	normal-leafed	2009	Grain	Czechia	Selgen	[2]
Rosakrone	s	normal-leafed	1970	Green fodder	Germany	Kruse	[6]
RU 165	s	normal-leafed	1945	Grain	Russia	IPK Gatersleben	[5]

^a Breeding lines

[1] (Federal Plant Variety Office, 2017)

[2] (European commission, 2017)

[3] (CPVO, 2021)

[4] (Annicchiarico & Filippi, 2007)

[5] (Genbankinformationssystem (GBIS) IPK, 2017)

[6] (Federal Plant Variety Office, 1997)

4.2.2 Molecular markers

The material was genotyped with the GenoPea 13.2K SNP Array chip (Tayeh et al. 2015a) by the company Trait Genetics, Gatersleben, Germany. The company received seeds. DNA extraction and SNP analyses were performed according to the standard protocols of the company.

4.2.3 Filtering

Loci with minor allele frequency (MAF) lower than 0.05 were removed. Markers with missing data were arranged as minor frequency alleles. Finally, 11,069 high quality markers were used for the analysis.

4.2.4 Data Analysis

Principal component analysis was calculated using *adeigenet* (Jombart et al. 2021), *poppr* (Kamvar et al. 2014), *factoextra* (Kassambara and Mundt 2020), *adegraphic* (Dray et al. 2018), *ape* (Paradis et al. 2021) packages for R software (3.6.2 version).

A K-means clustering approach (Hartigan and Wong 1979) was used to determine the most likely number of groups. Genetic distances were computed according to Nei's Genetic Distance (Nei 1972), and a dendrogram with bootstrap values was created by using *ape* and *poppr* packages for R software. The number/cycle of bootstrap was set to 1,000. Ward's minimum variance method, in which dissimilarities are squared before clustering (Ward.D2) (Murtagh and Legendre 2011) was used as the algorithm of clustering analysis.

The R syntaxes for data analysis are presented in the Supplementary Information.

4.3 Results

Principal component (PC) analysis for 46 pea genotypes illustrates that the first two PCs (PC1, PC2) explained about 20.5% of the total variation, with the first component explaining 14.2% and the second component explaining 6.3% (Figure 4.1).

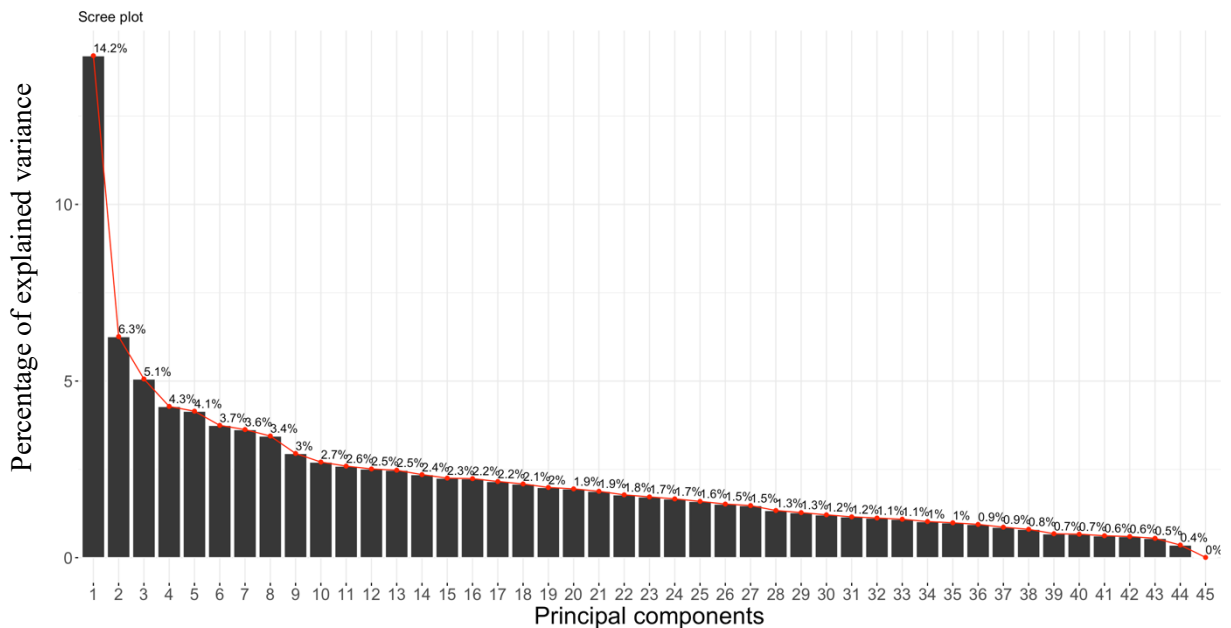


Figure 4.1 Principal component analysis of 46 pea genotypes by SNP markers, percentage of explained variance by each PC.

The first principal component in (Figure 4.2) separated normal-leafed and semi-leafless genotypes. The normal-leafed cultivars *Natura*, *Protecta*, *Bohatyr* and *Grana* are present in the semi-leafless group. For the second principal component, the two leaf types are not distinct. Both groups of leaf types have some genotypes (*AF447*, *AF448*, *GR409*, *GR 293EW*, *Pandora* of normal-leafed, and *Specter*, *James*, *Myster* of semi-leafless) which were located far from the position of their main groups. All these are winter cultivars, except *AF447* and *AF448*.

For the 46 pea genotypes, two main clusters were revealed by the dendrogram using Nei's genetic distance (Figure 4.3). This number of clusters is in correspondence with the result of the K-mean analysis (Supplementary Information, Figure 4.4). The assignment of the genotypes to the groups essentially agrees with the allocation made by principal component

analysis (Supplementary Information, Table 4.2). The upper cluster represents normal-leafed genotypes while the lower cluster mainly contains semi-leafless genotypes. The upper normal-leafed group contains four semi-leafless genotypes (Radley, KA-L11, KA-258, Specter), while the lower semi-leafless group contains four normal-leafed accessions (Grana, Natura, Bohatyr, Protecta). The mean genetic Nei's distance between the two groups is about 0.7. The sub-clusters in each group were determined with quite low bootstrap values (38.6% for both groups of leaf type). The bootstrap values of the following sub-clusters in the semi-leafless group are generally much higher than in the normal-leafed group and the distance between semi-leafless genotypes is in most cases clearly smaller than between normal-leafed genotypes.

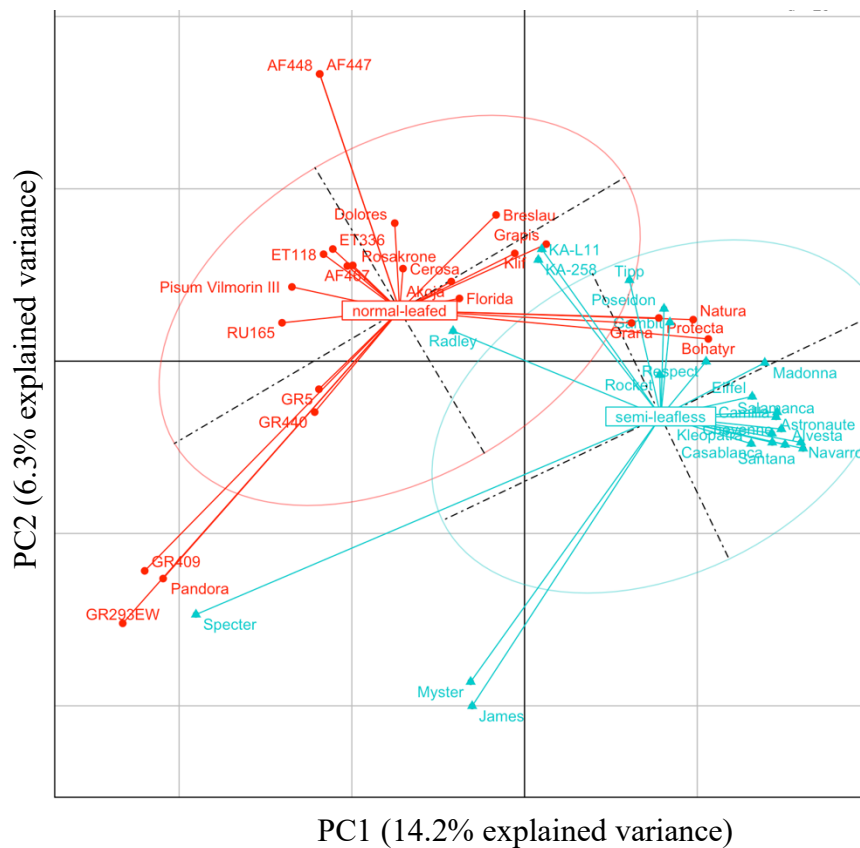


Figure 4. 2 The first two principal components of a principal component analysis of 46 pea genotypes by SNP markers (round shape, red colour: normal-leafed; triangle shape, cyan color: semi-leafless).

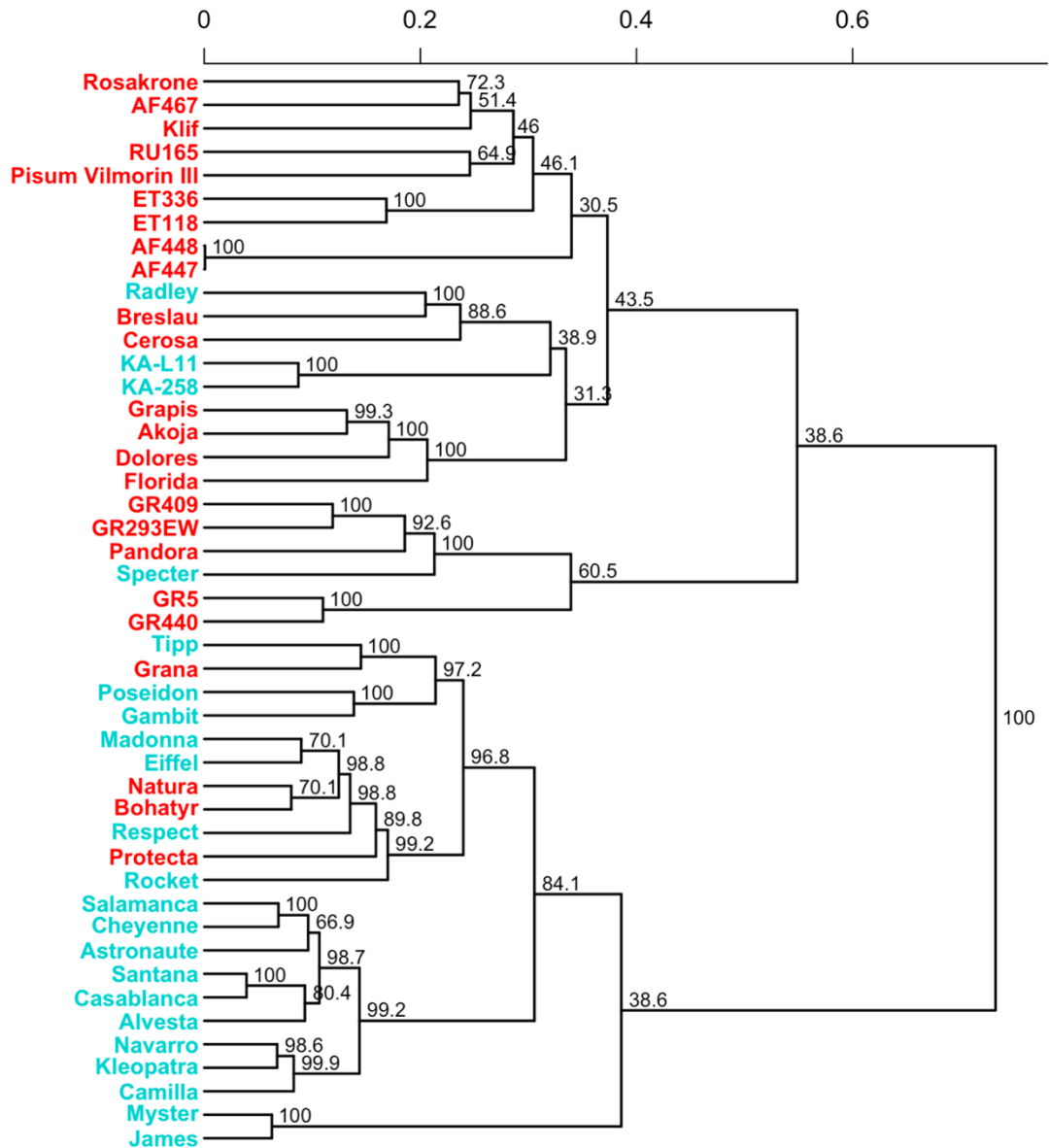


Figure 4.3 Dendrogram with bootstrap values for 46 pea genotypes. The X axis presents Nei genetic distances Red-labels represent normal-leaved genotypes, and cyan-labels represent semi-leafless genotypes.

4.4 Discussion

Our hypothesis was that normal-leafed and semi-leafless breeding materials would form two distinct groups with a clear genetic distinction, even though the phenotypic difference in leaf type is due to only one gene (Mikić et al., 2011). If confirmed, this hypothesis implies that older normal-leafed cultivars may therefore be an interesting genetic resource for breeding new semi-leafless cultivars.

For our study we used 46 pea genotypes. These are mainly European summer peas. But some winter peas and some accessions from Afghanistan and Ethiopia were also included. This is a good basis for assessing the relationship between leaf type and genetic diversity. With a much larger number of genotypes, it would have been possible to study marker-trait associations as well. However, this was not our goal, as such studies are available in large numbers (for review see Sharma et al. 2020).

The allocation of 46 pea genotypes in principal component analysis was generally in agreement with the results of clustering by the K-mean method and the dendrogram based on Nei's genetic distance. The dendrogram shows two very clearly separated clusters, one made up of mainly semi-leafless genotypes, the other one made up of mainly normal-leafed genotypes. The genetic distance between the two leaf types obviously reflects the breeding history of the cultivars, since in current breeding programs semi-leafless cultivars are normally developed from crosses between other semi-leafless cultivars. This is clearly demonstrated by the cluster of present European semi-leafless cultivars from different breeding companies with rather limited diversity (Figure 4.3: Salamanca, Cheyenne, Astronaute, Santana, Casablanca, Alvesta, Navarro, Cleopatra and Camilla). Baranger et al. (2004) and Tar'an et al. (2005) also observed that the genetic diversity within recently released pea cultivars in Western Europe is low, especially in the spring-sown feed pea breeding pool. The normal-leafed materials have a larger diversity including the cultivars from Russia, Afghanistan and Ethiopia.

Our results are in agreement with other studies: Baranger et al. (2004) used 121 protein- and PCR-based markers (RAPD, SSR, STS, Isozymes) to analyse the genetic relationships in a collection of 148 *Pisum* genotypes. Ward hierarchical classification based on Jaccard's distances revealed that almost all normal-leafed and semi-leafless genotypes were distributed in different groups, however some of them were also in the same sub-groups.

Some winter peas were grouped within spring sown cultivars, although there is generally a genetic distance between winter and spring sown genotypes.

Tar'an et al. (2005) used ISSR, RAPD, and SSR markers for genotyping 65 pea cultivars from North America, Europe, and Australia and 11 wild *Pisum* accessions. The results of PCA and the cluster analysis were generally consistent, in which the majority of the varieties that were bred in Europe and many Canadian varieties were separated from those that were developed in Australia and the USA, but clustered together into one large group far from groups of wild-type accessions. Leaf types were generally not clearly separated, but a small group of cultivars released before 1991 contained mainly normal-leafed genotypes. Jain et al. (2014) used 42 molecular markers (SSR and EST-derived markers) with 108 alleles to assess genetic diversity and population structure among 96 pea cultivars. The results showed that the effect of leaf type or geographical origin was faintly visible, and the dendrogram also illustrated normal-leafed cultivars were allocated scattered among semi-leafless genotypes in all three main clusters. The first cluster was dominated by the cultivars that had similar geographical origin and plant characteristics, while the second and the third cluster were a mixture of varieties with yellow and green seed coat of different geographical origin. That genotypes from different geographic origins are grouped together was explained by the exchange of germplasm among different countries, suggesting that these varieties may share common parents.

Our results show a few interesting exceptions to the otherwise clear clustering into semi-leafless and normal-leafed cultivars. The cluster of semi-leafless cultivars (lower part of the dendrogram in Figure 4.3) contains four normal-leafed cultivars. They all come from Czech breeding programs, in which the two leaf types apparently are not as strongly separated from one another as in breeding programs in other countries. In the normal-leafed cluster (upper part in Figure 4.3) there is a group of winter cultivars (GR409, GR293EW, Pandora) also containing the semi-leafless winter cultivar Specter, because in breeding of winter pea there was obviously not such a strong distinction between the two leaf types.

Simioniuc et al. (2002) used 20 RAPD primers with 175 polymorphic bands and 11 AFLP primer pairs with 462 polymorphic bands for genotyping 21 pea cultivars which were 16 semi-leafless and 5 normal-leafed. The UPGMA cluster analysis based on genetic similarity revealed two main groups, in which two normal-leafed genotypes are forming the first group, while the second one included all semi-leafless genotypes plus the three remaining normal-leafed genotypes. The normal-leafed cultivar Bohatyr and the semi-leafless cultivar Eiffel

are clustered closely together, as is also the case in our results (Figure 4.3). In this case the explanation is obvious: Bohatyr is one of the parents of Eiffel (Simioniuc et al. 2002). The other exceptions to the clear separation of the two leaf types can probably be explained in a similar way.

Ahmad et al. (2015) observed that cluster analysis classified pea genotypes into groups that were somewhat consistent with their geographical origins with some exceptions, that may have been introduced from other regions by germplasm exchange programs. Jing et al. (2010) observed a clear separation between wild and cultivated accessions, but the subgroups of cultivated peas did not coincide well with the country of origin. Rana et al. (2017), when analyzing a worldwide collection of 151 accessions, found three separate groups, that were not in close agreement with different geographic origins.

In our material, three accessions originate from Afghanistan and two from Ethiopia (Table 4.1). Two accessions from Afghanistan (AF447 and AF448) have extreme values for the second PC in the PCA (Figure 4.2). In the dendrogram, however, the five accessions from Afghanistan and Ethiopia form one normal-leafed cluster together with four other accessions from Europe (Figure 4.3). The “exotic” accessions probably were introduced from Europe in the past, especially the genotypes from Ethiopia.

The low diversity of European pea varieties was mentioned by Baranger et al. (2004) and Tar'an et al. (2005), suggesting that it may encourage breeders to include exotic material in their breeding programs. Our results showed a larger genetic distance between genotypes in the normal-leafed group compared to semi-leafless genotypes. Probably all semi-leafless cultivars carry the same original *afila* mutation. We suggest, that a first step to increase genetic diversity should be to include normal-leafed pea genotypes as an interesting genetic resource, even when breeding semi-leafless cultivars.

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Supplementary

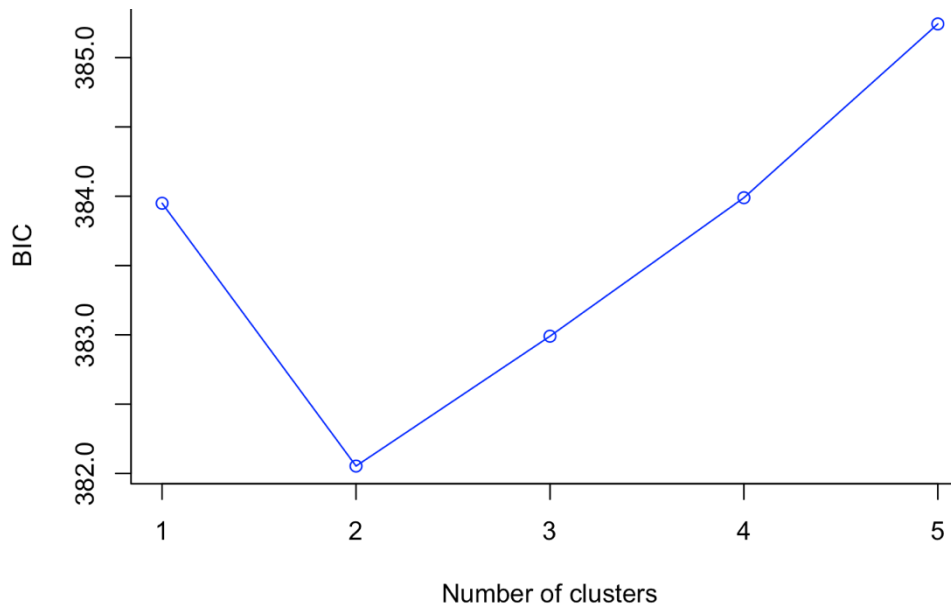


Figure 4.4. K-means optimal clusters determination. The lowest Bayesian Information Criterion (BIC) indicates two clusters as the best fit model

Table 4.2 Allocation of pea genotypes by K-mean method

Genotypes	Group	Genotypes	Group
Alvesta	1	AF447	2
Astronaute	1	AF448	2
Bohatyr	1	AF467	2
Camilla	1	Akoja	2
Casablanca	1	Breslau	2
Cheyenne	1	Cerosa	2
Eiffel	1	Dolores	2
Gambit	1	ET118	2
Grana	1	ET336	2
KA-258	1	Florida	2
KA-L11	1	GR293EW	2
Kleopatra	1	GR409	2
Madonna	1	GR440	2
Natura	1	GR5	2
Navarro	1	Grapis	2
Poseidon	1	James	2
Protecta	1	Klif	2
Respect	1	Myster	2
Rocket	1	Pandora	2
Salamanca	1	Pisum Vilmorin II	2
Santana	1	Radley	2
Tipp	1	Rosakrone	2
		RU165	2
		Specter	2

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Chapter 5

General Discussion

5.1 Performance of different leaf types in pea

When comparing diverse cultivars with different genetic background, pea genotypes of normal-leafed type were lower in average seed dry yield and straw dry yield, but higher in nitrogen content in seed and nitrogen content in straw compared to semi-leafless cultivars. For the traits nitrogen yield in seed and nitrogen yield in straw, semi-leafless cultivars with the advantages of higher yield are on average superior to normal-leafed peas (Chapter 2). These results are in agreement with some previous studies (Acikgoz et al., 2009; Čupić et al., 2013; Singh & Srivastava, 2015). However, normal-leafed peas have been recorded to be superior to semi-leafless peas by Turk & Albayrak (2012), Pesic et al. (2013), and Olle (2017; 2019). The difference in grain yield between semi-leafless and normal-leafed pea cultivars mainly come from the fact that today almost all breeding programs only concentrate on semi-leafless peas to exploit their lodging resistance. Consequently, the yield of modern semi-leafless cultivars is improved rapidly. In addition, some other studies even pointed out that under drought conditions both leaf types have similar performance (Martin et al., 1994), or that the difference in grain yield between the semi-leafless and normal-leafed peas was not significant (Mihailović et al., 2004; Mikić et al., 2006). In our research material, most of the normal-leafed cultivars were much older than the semi-leafless cultivars, in which some normal-leafed accessions were released even in the years of 1940s, while almost all semi-leafless genotypes were developed in 2000s (Chapter 2, Table 2.1). Therefore, the differences between the two leaf types based on these research materials could not reveal the potential of each leaf type in production and breeding.

On the other hand, in the comparison between the two leaf types within a similar genetic background, the superiority of the semi-leafless group can no longer be observed (Chapter 3). The average seed dry yield of the normal-leafed bulks is clearly higher than that of semi-leafless bulks, though the average straw dry yield is still slightly lower than that of semi-leafless peas. Especially, the difference in nitrogen content in seed and nitrogen content in

straw between the two leaf types is not as large as in the comparison of diverse genotypes. Because of the higher seed dry yield of normal-leafed peas in the second trial, the nitrogen yield in seed is also higher than that of semi-leafless peas. In this experiment, the bulks which represent the two leaf types are in the same generation from the crosses between normal-leafed and semi-leafless parents. They were not under any pressure of artificial selection on yield, diseases resistance, or abiotic stress tolerance. The comparison of such near-isogenic bulks which only differ in leaf type could reveal fully the performances of each leaf type, which was not met in the previous reports. Only very few results of the effect of the *af* mutant in genetically comparable material had been published. They also observed the superiority of the normal-leafed type to the semi-leafless type (Goldman & Gritton, 1992; Stelling, 1994; Pesic et al., 2013).

The mean values of the important agronomic traits of two leaf types in the two sets of research materials are presented in Table 5.1. In agreement with previous studies, the normal-leafed genotypes revealed lodging after the flowering stage and even more seriously at the stage of pod maturity in both experiments with level of 7 to 8 (Table 5.1). However, the semi-leafless cultivars also lodged slightly at levels of 2 to 3 (Chapter 2, and Chapter 3). The effects of lodging in normal-leafed peas have been reported in many studies. The consequence could be the reduction of seed yield, and low quality of straw, due to less sunlight and high moisture condition which is favorable for the development of diseases in the lower parts after the plant lodged. Regarding semi-leafless peas the plant structure with robust tendrils supports a stable standing ability, however some tall semi-leafless genotypes also showed slightly lodging at the maturity stage.

Table 5.1 Mean values of the most important agronomic traits of two leaf types in two different sets of research materials.

Traits	Diverse genotypes (ch. 2)		Near-isogenic bulks (ch. 3)	
	Normal-leafed	Semi-leafless	Normal-leafed	Semi-leafless
Lodging	7.86	2.41	7.15	2.67
Seed dry yield (t/ha)	1.64	2.48	2.16	1.72
Straw dry yield (t/ha)	2.55	3.57	2.70	2.83
Nitrogen content in seed (%)	3.91	3.60	3.42	3.40
Nitrogen content in straw (%)	1.30	1.03	1.08	1.17
Nitrogen yield in seed (kg/ha)	63.10	89.63	74.14	58.90
Nitrogen yield in straw (kg/ha)	33.51	35.38	29.75	33.11

Compared to semi-leafless cultivars, the leaflets of the normal-leafed genotypes result in higher ability of soil covering or light interception which is one of the important traits in breeding for organic production. Pea and cereals in mixtures have been traditionally used in many parts of the world. This solution also could reduce the lodging problem of normal-leafed peas.

5.2 Genetic distance between different leaf types in pea

The first component of the principal components analysis of 54 genotypes based on 14 morphological and agronomic traits explained about 55% of the variation, and separated the two leaf types with some exceptions for both groups. The semi-leafless cultivars “Specter” and “Radley” are displayed in the normal-leafed group, while some normal-leafed genotypes as “Bohatyr”, “Natura”, “Protecta”, “Grana”, “Grapis” appeared in the semi-leafless group (Figure 5.1 a). Similarly, the first component of the principal component analysis of 46 pea genotypes based on the results of genotyping with SNP markers also split up the two leaf types (Figure 5.1 b). In the second component, the winter peas which consists of normal-leafed genotypes (Specter, Myster, James) and semi-leafless cultivars (GR409, GR293EW, Pandora) displayed as a particular group separated from the main group of both leaf types.

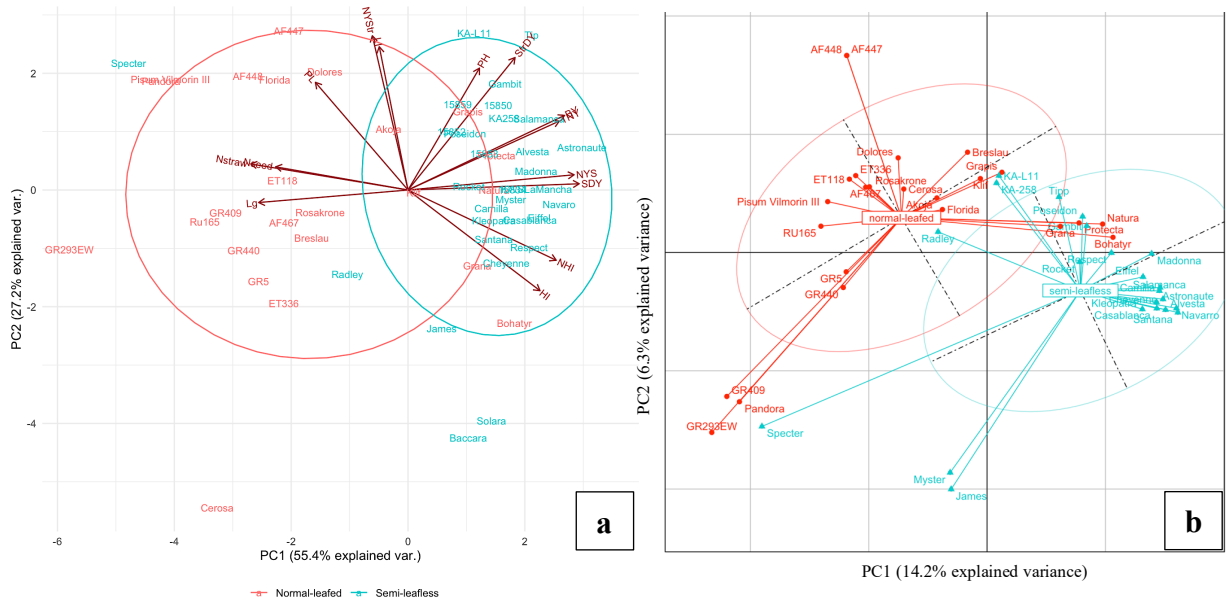


Figure 5.1 The first two components of principal component analysis based on a) 54 pea genotypes and 14 morphological and agronomic traits (Chapter 2); and b) 46 pea genotypes screened by SNP molecular markers (Chapter 4).

Nevertheless, there are differences between the two principal component analyses, as the first analysis was based on phenotypic traits which are influenced by the different environments. That could be the reason that the sub-group of winter peas which included the genotypes of both leaf types such as “GR293EW”, “GR409”, “Pandora”, “Specter”, and “James”, “Myster” was established only in the second analysis (Figure 5.1b). The genotypes “AF447” and “AF448” are at the same location by SNP marker genotyping whereas they are slightly distinct based on the morphological traits.

5.3 General conclusions

Today, semi-leafless peas with higher yields and the advantage of lodging resistance (Chapter 2) are the dominant leaf type in production. But with a comparable genetic basis, normal-leafed peas have a higher seed yield potential, which however cannot be realized in practical production, due to severe lodging (Chapter 3).

In general, both phenotypic studies (Chapter 2) and molecular marker data (Chapter 4) show, that normal-leafed and semi-leafless peas can be considered as two gene pools. Therefore, normal-leafed peas with their broader genetic diversity are an important genetic resource also for breeding semi-leafless peas.

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Summary

Pea (*Pisum sativum* L., $2n = 14$) is the most important cool season grain legume in Europe. Peas are mainly used as protein-rich feed for animals as an alternative to soybeans and faba beans. There is a special interest in peas in organic agriculture also because of their high value as a pre-crop due to the nitrogen fixation by symbiotic rhizobia and the high nitrogen content in the residues. For a long time, the main limitation for pea production was the high susceptibility to lodging of the normal-leafed type and consequently severe problems with harvesting. A semi-leafless mutant reduced this problem, but the lack of leaflets may have impaired productivity. The aim of this study was to compare the two leaf types with respect to the differences in yield and nitrogen content, and to identify genotypes that could be a resource for combining a high protein content in seed with a high nitrogen yield in the straw. The benefit of this study is to help increasing the pre-crop value of pea and to broaden its genetic diversity for breeding programs. The thesis consists of three different experiments.

In the first experiment, 24 normal-leafed and 30 semi-leafless pea genotypes were evaluated in randomized complete block design with two replicates in three environments in 2017 and 2018 near Göttingen, Germany. The materials were recently released to very old cultivars, as well as recent breeding lines and some genetic resources, collected from gene banks, seed companies and breeders in Europe. Fourteen important morphological and agronomic traits were measured: light interception, lodging, plant length, plot height, seed dry yield, nitrogen content in seed, nitrogen yield in seed, straw dry yield, nitrogen content in straw, nitrogen yield in straw, biomass yield, total nitrogen yield, harvest index, and nitrogen harvest index.

For the comparison of semi-leafless and normal-leafed peas under comparable genetic backgrounds, in the second experiment near-isogenic bulks were established for four crosses. The crosses were performed in 2013 -2014 with parents that are different in leaf type (normal-leafed and semi-leafless) and plant length (long and short), and lines in F4, F6 were developed, respectively. In each cross, four bulks of 10 segregating lines each were bulked which are the combination of different leaf type and plant length: semi-leafless, short; semi-leafless, long; normal-leafed, short; normal-leafed, long. These four bulks of each of the four crosses were tested in four environments in 2018 and 2019 for the traits: light interception, plant length, plot height, lodging, seed dry yield, straw dry yield, nitrogen content in seeds,

nitrogen content in straw, nitrogen yield in seed, nitrogen yield in straw, biomass yield, total nitrogen yield, harvest index and nitrogen harvest index.

In the third experiment, a collection of 46 pea genotypes from the initial diversity trial were analysed for genetic diversity using the GenoPea 13.2K SNP Array chip by the company Trait Genetics.

In the first experiment, seed yield and straw yield were higher for semi-leafless genotypes (51% and 40%, respectively, compared to normal-leafed genotypes). However, nitrogen content in seed and nitrogen content in straw were lower for these genotypes (10% and 30%, respectively). A negative correlation of seed yield with nitrogen content in seed was observed in both normal-leafed and semi-leafless peas with correlation coefficients (r) of -0.67 and -0.36, respectively. A similar relationship was also recorded between straw yield and nitrogen content in straw ($r = -0.48$ for normal-leafed, and $r = -0.30$ for semi-leafless peas). However, there was no correlation between nitrogen yield in seed and nitrogen yield in straw. In addition, semi-leafless peas were significantly higher than normal-leafed genotypes in total nitrogen yield, biomass yield, plot high, and nitrogen harvest index. However, most normal-leafed genotypes in this experiment were released earlier than semi-leafless genotypes, and some of them even were genetic resources not adapted to European climate. Some recent normal-leafed cultivars reached the yield level of semi-leafless genotypes. The lodging scores of normal-leafed genotypes were on average 7 to 9 (1 = no lodging, 9 = total lodging), whereas this value of semi-leafless accessions were just between 2 and 3. The first component of a principal components analysis (PCA) separated the two leaf types with some exceptions. It was positively influenced by the traits seed dry yield, nitrogen yield in seed, harvest index, nitrogen harvest index, total nitrogen yield, biomass yield, with higher values for the semi-leafless group, while the traits lodging, nitrogen content in seed and nitrogen content in straw had a negative impact, with higher values for the normal-leafed group.

The results of the second experiment illustrate that normal-leafed peas were about 25% higher in seed dry yield although seriously lodging, while the semi-leafless type was almost 5% higher in straw dry yield, however about 6% higher biomass yield was recorded for normal-leafed peas. The average difference of nitrogen content in seed and in straw between the two leaf types was small but significant. Normal-leafed peas were about 26% higher in nitrogen yield in seed, but approximately 11% lower in nitrogen yield in straw compared to the semi-leafless group. In the principal components analysis, the first component explained

about 48% of the total variance, and also distinguished between the two leaf types. Normal-leaved peas had higher values for harvest index, nitrogen harvest index, seed dry yield, nitrogen yield in seed, light interception, total nitrogen yield, and nitrogen content in seed.

In all field experiments, the light interception of normal-leaved peas is always significantly higher than that of semi-leafless peas, 83.5% vs 81.1% in the first experiment, and 84.2% vs 78.4% in the trial of bulks of leaf types, respectively.

The genotyping by SNPs markers of 46 accession resulted in a principal component analysis where the first two principal components explained about 20.5% of the total variation. The first component explaining 14.2% and separated the research materials in the two groups semi-leafless type and normal-leaved type, whereas the second component, which explained 6.3% of variance reveal a small outlier group which consists of the winter peas from both leaf types, and two genotypes from Afghanistan at similar position. A dendrogram with bootstrap values using the genetic distance between the 46 pea genotypes illustrated also two groups of genotypes mainly corresponding to the two leaf types. There are a few interesting exceptions from the clear clustering into semi-leafless and normal-leaved cultivars. The cluster of semi-leafless cultivars contains four normal-leaved cultivars. In the normal-leaved cluster, there is a group of winter cultivars (GR409, GR293EW, Pandora) also containing the semi-leafless cultivar Specter, because in breeding of winter pea there was obviously not such a strong distinction between the two leaf types. Some new semi-leafless cultivars from Europe such as Salamanca, Cheyenne, Astronaute, Santana, Casablanca, Alvesta, Navarro, Cleopatra and Camilla were assigned together in a sub-group. In general, semi-leafless peas have a lower genetic distance to each other than the normal-leaved peas.

The results of the present study demonstrate the role of normal-leaved pea cultivars in breeding, especially for low-input production systems. The absence of a negative correlation between nitrogen yield in seed and in straw indicates that a high protein yield can be combined with a high pre-crop value. The value of pea in crop rotations can be increased by using superior cultivars and by breeding for both traits. Although seriously lodging, normal-leaved genotypes still have a potential of high yield and protein content especially for intercropping and organic agriculture. Due to the large distance between the two leaf types, normal-leaved peas are an interesting resource to increase the genetic diversity even when breeding semi-leafless peas.

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