

VEGETABLES II

HANDBOOK OF PLANT BREEDING

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Volume 1

Vegetables I: Asteraceae, Brassicaceae, Chenopodiaceae, and Cucurbitaceae

Edited by Jaime Prohens and Fernando Nuez

Volume 2

Vegetables II: Fabaceae, Liliaceae, Solanaceae and Umbelliferae

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Fabaceae, Liliaceae, Solanaceae, and Umbelliferae

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Cover illustration: Typical seed production field for an extremely early Japanese onion cultivar (courtesy of M. Shigyo)

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Preface

The production and consumption of vegetables has expanded dramatically in the last years, with a global growth in the production of more than 50% in the last decade, a rate of increase that is much higher than for other plant commodities. Vegetables constitute an important part of a varied and healthy diet and provide significant amounts of vitamins, antioxidants and other substances that prevent diseases and contribute to an improvement in the quality of life. In consequence, it is expected that in the coming years, vegetable crops production will continue its expansion.

Improved varieties have had a main role in the increases in yield and quality of vegetable crops. In this respect, the vegetables seed market is very dynamic and competitive, and predominant varieties are quickly replaced by new varieties. Therefore, updated information on the state of the art of the genetic improvement of specific crops is of interest to vegetable crops breeders, researchers and scholars. During the last years an immense quantity of new knowledge on the genetic diversity of vegetables and the utilization of genetic resources, breeding methods and techniques, and on the development and utilization of modern biotechnologies in vegetables crop breeding has accumulated, and there is a need of a major reference work that synthesizes this information. This is our objective.

The diversity of vegetable crops is appalling, with hundreds of species being (or having been) grown. However, among this plethora of crops, there are some which are prominent, and for which there has been a greater development in the breeding science and development of varieties. In consequence, we have produced two volumes devoted to 20 of these most important vegetable crops. These crops belong to eight different botanical families. Because in many cases crops from the same botanical family share many reproductive, physiological, and agronomic features, as well as similar breeding techniques, we have decided to group them by this taxonomic category. In this respect, this second volume includes 8 chapters that deal with vegetables that belong to four families: Fabaceae or Leguminosae (garden pea, and snap bean), Liliaceae (asparagus, and onion), Solanaceae (eggplant, pepper, and tomato) and Umbelliferae or Apiaceae (carrot).

Chapters have been written by outstanding breeders with wide experience in the crop treated. Each chapter includes information on the origin and domestication, varietal groups, genetic resources, major breeding achievements and current goals of breeding, breeding methods and techniques, integration of the new biotechnologies in the breeding programmes, and the production of seed of specific crops.

The completion of this book would not have been possible without the contributions of the many authors, who have devoted much time to the task of writing the chapters. We also want to thank the staff of Springer, in particular Jinnie Kim and Shoshana Sternlicht, who have made possible to produce a high quality book in a very short time span. We are also indebted to many colleagues for useful suggestions that have contributed to improve this book.

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Family Fabaceae (=Leguminosae)

Garden Pea

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1 Introduction

The variability of the garden pea (*Pisum sativum* L.) and the variety of forms in which it is consumed are a testimony to its long history of cultivation, adaptability and popularity as a crop in countries around the world. The different crop forms are based on different harvest times during the development of either the fruit or the embryo and the presence of particular gene combinations characterize the market product. Those relating to the embryo are those of the fresh vegetable or picked pea, canned, frozen and dehydrated or freeze dried pea markets (Fig. 1 a-d), while those associated with the immature pod are the snow, sugar or mangetout and the sugar snap types (Fig. 1, e and f).

When harvested as young immature embryos while liquid endosperm is still present, peas are rich in vitamins and sugars and appeal to people of all ages. This stage of development is of relatively short duration and is only achieved in large quantities with successional sowings of a single variety or by simultaneous sowing of varieties with staggered flowering times. Both these strategies are utilized by the vining industry, the produce of which is found in both canned and frozen forms where the peas are often graded and of very uniform size. Once embryos have past this stage, they enter the phase of storage product accumulation where starch and proteins are laid down and the levels of sugar decrease. Peas harvested during this phase, when the pod is starting to show signs of starting to dry, are consumed either as fresh vegetable peas or are dehydrated (via either hot-air drying or freeze drying) and used in soups, snacks and other fast foods. Following this stage the accumulation of storage products continues, the embryo starts to lose fresh weight and enters the maturation phase ultimately leading to a dry seed. The dried seed form *per se* is not considered a vegetable but an arable or combinable crop and will not be covered explicitly in this chapter. Being the same species, there are naturally many issues that

are common to both the vegetable and combined crop and references to the latter are mentioned by way of contrasting the two forms and highlighting the differences.

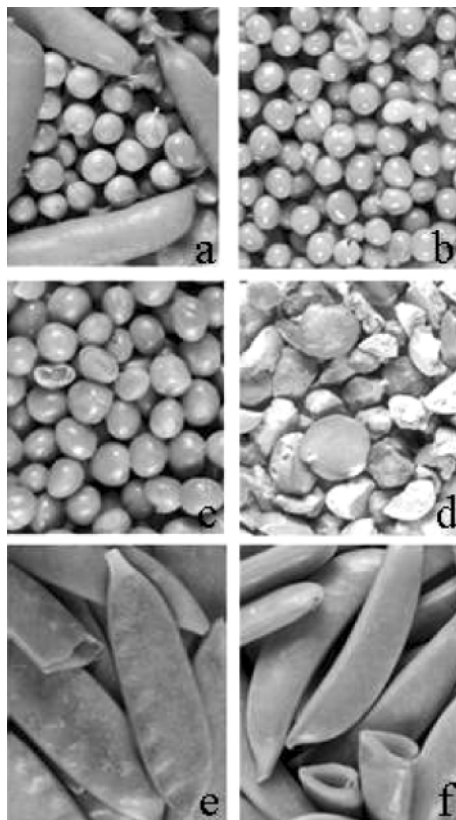


Fig. 1. Forms of vegetable peas. a. fresh picked, b. canned, c. frozen, d. dehydrated, e. mangetout, and f. snap.

References in early herbals (Gerard, 1597) demonstrate that garden peas were a well established by the 1500's and considered something of a premium, particularly the early crops, which are cited as having been transported long distances at great cost to the dining tables of the gentry. There were already a range of distinct plant types at this time 'differing very notably in many respects including 'pease without cods', now referred to as snow peas or mangetout type that were eaten whole and tufted or crowned peas so called because the pods were clustered at the top of the plant rather than in the middle that we now know is as a result of the strong expression of apical fasciation. The popularity of the pea continued and by the end of the 1800's, the vegetable seed list of Sutton's starts with 15 pages devoted to 44 different pea varieties of all classes, illustrating how diverse and popular peas were

within kitchen gardens (Sutton's, 1899). Their persistent popularity even today can be attributed to their relative ease of cultivation and storage, their extended period of harvesting and their taste when freshly picked and cooked. These qualities are appreciated across all cool temperate regions of the world. It is interesting to note that the large-scale production of vegetable peas for the international market which aims to deliver fresh vegetable such as peas to the consumer all year round, is still based largely on old varieties from the early part of the 19th century and is a testament to the variation that has been maintained in cultivation. A wide range of cultivated forms of pea can still be found growing today in gardens and small-holdings in many different parts of the world. Many of these still represent ancient lineage and possible sources of adaptive variation.

2 Origin and Domestication

Pea is an old world cool season annual legume crop whose origins trace back to the primary centre of origin in the near and middle east. Carbonised remains of pea have been found at neolithic farming villages in northern Iraq, southern and south eastern Turkey and Syria and indicate their cultivation and use as food as early as 7000-6000 BC. Their presence is found in remains at sites in Southern Europe soon after (Zohary and Hopf, 1973). While it cannot be proved, it is highly likely that they were consumed in both a fresh vegetable as well as cooked forms. An important secondary centre of diversity for pea is the highland Asiatic region of the Hindukusch that runs the whole length of the southern slopes of the Himalyan mountain range. Distinct forms of cultivated pea from this region include the distinct long vined 'afghan' type and the shorter statured Tibetan ecotype grown on agricultural terraces at high altitudes. Interestingly, examination of germplasm from this region and neighbouring lowland production areas shows clear evidence of the introgression of morphological characters from the high altitude region. The endemic forms of pea from the Transcaucaia and Volga region are a very distinct type with fine foliage and very small seeds (60-80 mg) and are still recognised by some as a separate sub species, *Pisum sativum* spp. *transcaucasicum* (Govorov, 1937). A further secondary centre of diversity includes the central highland region of Ethiopia and uplands of Southern Yemen, which covers the currently known distributional range of *Pisum sativum* ssp. *abyssinicum*. The taxon is well described and distinct from all other *Pisum sativum* forms for a range of morphological characters. Molecular diversity studies have confirmed the narrow genetic variation within known germplasm of this form but also its distinctness from all other sativum forms and postulated its existence as an independent domestication event to that of *Pisum sativum* (Lu et al., 1996; Vershinin et al., 2003). Important novel allelic variation has already been identified within abyssinicum material and this distinct gene pool is currently being explored by groups undertaking wide crosses, mapping and the production of recombinant inbred populations (Weeden et al., 2004).

In these secondary centres are found various forms that demonstrate the wide adaptability of peas to changes in habitat. Numerous expeditions in the 1900's to collect herbarium specimens and germplasm resulted in a wealth of material that

breeders and researchers have been scrutinizing for years. One of the most detailed and authoritative accounts of the genus and classification of forms studies *in situ* is that of Govorov (1937; Gentry, 1974).

The genus *Pisum* comprises of only a small number of taxa. Despite this, the taxonomic literature is far from clear at the level of rank. The most recent review of the *Pisum* taxonomy and ecogeography can be found in Maxted and Ambrose (2001). All taxa within *Pisum* are diploid ($2n=14$) and the majority are fully intercrossable with a few being more difficult but possible (Ben-Ze'ev and Zohary, 1973). The exact nature of the wild forms that were taken into cultivation and domesticated is impossible to establish unequivocally. Zohary and Hopf, (1973) postulate *Pisum humile* syn. *syriacum* as a possible candidate, as its form closely resembles that of cultivated forms. The evidence emerging from molecular studies has revealed the wide genetic variability within *Pisum elatius* across its distributional range and the presence of material exhibiting characteristics from both *elatius* and *sativum* forms supports the view that there has been frequent introgression between these forms and a better considered as a species complex (Vershinin et al., 2003).

A series of traits that have been associated with domestication are presented in Table 1. A number of these can be considered as prerequisite to the widespread adoption into agrarian practices are those of thin seed coat and non-dehiscent pods. Thin seed coats allows for rapid imbibition and results in more even germination and establishment while non-dehiscent pods, while not essential, as the plants could have been cut prior to full maturity and contained in such a way as to trap the seeds as the pods opened, would have greatly eased the handling and processing of the crop. Rough testa has been associated with domestication (Zohary and Hopf, 1973) but this is not categorical as there are numerous examples of cultivated forms with rough testa eg. Ghatt oasis and the Canary Islands (pers. obs.). The presence of such characters within cultivated material may help in unravelling some of the ancestral forms and lineages within pea germplasm. The seed size of cultivated material has been increased fourfold compared to that of wild material although there is overlap at between the two forms.

Table 1. Domestication traits in wild and cultivated pea and their genetic basis.

Trait	Wild type	Cultivated	Gene basis
Testa surface	Gritty/ rough	Smooth	<i>Gty</i>
Testa thickness	Thick, impermeable so slow to imbibe	Thin resulting in rapid imbibition	
Pod Dehiscence	Strongly dehiscent	Non-dehiscent	<i>Dpo</i>
Seed size (mg)	60-120	80-550	

Theories into the further spread of peas are gathered from archeological, ethnobotanic and botanical evidence. Along with other crops, their ease of storage, cooking and nutritional properties were key reasons that lead to peas being included in many expeditions and military campaigns and their early dispersal and uptake into other cool temperate regions of the world. The Greeks under Alexander the Great extended their empire eastwards into Mesopotamia and south into Africa and the Romans were responsible for the introduction of cultivated forms into Western

Europe along with many other crop species. The numerous expeditions in the 1900's to primary and secondary centres of origin to collect herbarium specimens and germplasm have resulted in a legacy for breeders and researchers (Gentry, 1974). It is clear however that there are still gaps in our knowledge and coverage and that new locations and populations of wild material are yet to come to light.

3 Varietal Groups

As noted previously, the variation within cultivated peas had been noted and described in numerous references and seed catalogues from the 1700's onwards which was also the time of increasing popularity of the crop. This in itself led to an explosion of new named forms coming to market of which there were clear indications that many were not new forms but just newly renamed. It was not until the early 1900's that systematic cataloguing of cultivated forms was undertaken. Some notable references of this period that detail the characteristics of many hundreds of different varieties and their groupings include the works by Hendrick (1928), Mateo Box (1955), Fourmont (1956) and Sneddon and Squibbs (1958). The primary characters used for grouping varieties relate to seed and pod types, maturity groups and height of the crop and reflected the variation across the market types. The number of groups for which keys were developed varied from anything from 18 to 36 different groups within which were numerous subgroups. Many of these characters are still in general use today but the emphasis is more on specific characters and the descriptor states which, wherever possible are linked to the allelic forms or combinations that underlies that character. A useful point of reference list of characters relating to variation within cultivated forms is the list used in the UPOV guidelines for *Pisum* (Table 2). These form the basis of the distinctness, uniformity and stability test that must be undergone as part of the plant variety rights system (UPOV) and while not covering all the primary characters cited earlier, focuses on those that are highly heritable. The key characters to note from table 2 concerning the various market types of pea are the presence of the *i* allele which results in the peas remaining green rather than their wild type status of yellow. The presence of the *r* and *rb* alleles results in the reduction in starch and higher concentration of sugars found in frozen peas. In the pod types, the presence of the recessive forms of *P* and *V* alleles result in the partial or complete loss of the inner sclerenchyma layer of the pod responsible for giving the pod wall rigidity. Loss of this layer underpins the snow pea or mangetout type. The presence of the recessive allele of the *N* locus results in a thickening of the middle cell layer of the pod resulting in the thicker crunchy textured pods of the sugar snap pea type.

New characters introduced into commercial cultivars since the 1970's represented in the UPOV list are associated with variation in stipule and leaf forms. The first of these is associated with narrow pointed stipules and leaflets that are characteristic of 'rabbit eared' or rouge forms. The genetics of the rogue syndrome which includes a none nuclear component are still not well understood and the character is presently confined to combined dried pea rather than vegetable type. Rogue off types of a range of old vegetable pea varieties have been observed and

isolated but the trait is not seen to offer any advantage to the vegetable pea market so will not be dealt with further. The leaf character that has become widely used in all form of pea breeding since the mid 1970's is the *af* gene (*af*) which converts leaflets into tendrils. This character is discussed in more detail in section 5.

Table 2. List of UPOV characters, phenotypic states and associated loci used for grouping varieties.

	Character	Descriptor states	Loci
Seed			
1	Shape of starch grain (cotyledonary character)	Round, wrinkled, dimpled	<i>R, Rb</i>
2	Cotyledon colour	Yellow, green, mixed Orange	<i>I</i> <i>Orc</i>
3	Testa marbling	Brown patterning	<i>M</i>
4	Testa anthocyanin	Violet or pink spots, stripes	<i>F, Fs</i>
5	Hilum colour	Cream, black	<i>Pl</i>
Plant			
6	Anthocyanin colouration	Purple, red to pink	<i>A, B, Am</i>
7	Leaf	Leaflets	<i>Af</i>
8	Stipule	Small or rudimentary	<i>St</i>
9	Stipule	Rounded apex, pointed	' <i>Rogue syndrome</i> '
10	Stipule	Flecked, non-flecked	<i>Fl</i>
Pod			
11	Pod wall parchment		<i>P, V</i>
12	Thickened pod wall		<i>N</i>
13	Shape at distal end	Blunt, pointed	<i>Bt</i>
14	Colour	Yellow Blue-green Purple	<i>Gp</i> <i>Dp</i> <i>Pu, Pur</i>
15	Intensity of green		<i>Pa, Vim</i>

While the UPOV list is useful, it only represents a key for grouping currently registered commercial varieties and so does not cover the wider variation within pea. Neither does it cover useful characters that are based on combinations of genes and an interaction with the environment and thus vary from year to year. The two other primary characteristics referred to earlier that fall into this category are plant height (associated with genes for internode length and an interaction with nodes to flower) and maturity groups (linked to flowering time). Both these characters present problems in quantifying them in practical terms and it is interesting to compare the findings presented in two of the classifications works namely those of Hendrick, (1928) and Sneddon and Squibbs (1958, table 3). Both systems are based on the records obtained for a large number of varieties grown over many years and in the case of Hendrick, many sites. Both reports detail four categories for plant height with Sneddon and Squibbs going so far as to quantify the range with respect to results obtained in one year and at one location. For maturity groups Sneddon and Squibbs

describe 6 categories whereas Hendrick uses only one (extra early) in his system although in the descriptions of many of the individual varieties, the terms second early and mid season are used.

Table 3. Height and maturity categories in two reference on pea cultivar classification.

	Height categories	Maturity categories
Hendrick (1928)	Very dwarf Dwarf Medium Tall	Extra early
Sneddon and Squibbs (1958) * results presented for 1953	Dwarf- under 45cm Dwarf-medium- 45-75cm Medium- 76-111cm Tall- >111cm	First early- 63 days* Early- 64-67 Second early- 68-71 Mid season- 72-75 Late- 76-79 Very late- 80+

While the UPOV guidelines are important in defining and describing the categories of pea that are cover the variation in commercial material, it is essential for anyone engaging with breeding to know the requirements of the market of their target countries. The registration requirements for peas vary from country to country. A useful survey of requirements across fifteen European countries for agronomic, processing and chemical classes of characters can be found in (Engqvist, 2001) and showed widespread differences for all characters in all classes.

4 Genetic Resources

The inbreeding nature and diploid status of peas and the ease of maintaining fixed inbred lines, together with their wide spread popularity and cultivation have all contributed to the wealth of genetic resources that have been developed associated with pea. This section reviews the current status and recent developments in pea genetic resources that are available within the public domain

A large number of *ex situ* germplasm collections for pea exist around the world (Table 4). Historically, these were established to provide access to a range of variation from the centres of diversity and different gene pools for taxonomic reference, research and to underpin breeding programs. These *ex situ* collections have a long history of active collaboration between each other and in supporting wider initiatives (Ambrose and Green, 1991). A working group for grain legumes exists as part of the European Cooperative Programme for Crop Genetic Resources which brings together the formal and informal sectors to collaborate on activities and initiatives of common interest such as the European central crop databases (ECP/GR). In the absence of a CGIAR institution with a global mandate for pea, an international consortium for pea genetic resources (PeaGRIC) has recently been formed that links together key collections within Europe, USA, ICARDA and

Australia. The aims of the consortium will be to coordinate pea genetic resources in the broadest sense and to provide stakeholder groups with a readily identified body with which they can interact. To this end two of the primary objectives of the consortium are to draw together key information resources and initiate the formation of a decentralised international core collection out of the many individual core collection initiatives.

Table 4. *Ex situ* germplasm collections of *Pisum* with holdings in excess of 1000 accessions.

FAO Institute code	Country	Number acces- sions	Web site for Germplasm searches
ATFC	Australia	6567	http://www2.dpi.qld.gov.au/extra/asp/AusPGRIS/
SAD	Bulgaria	2787	http://www.genebank.hit.bg/
ICAR-CAAS	China	3837	http://icgr.caas.net.cn/cgris_english.html
GAT	Germany	5336	http://fox-serv.ipk-gatersleben.de/
BAR	Italy	4297	http://www.ba.cnr.it/areagg34/germoplasma/2legbk.htm
CGN	The Netherlands	1008	http://www.cgn.wur.nl/pgr/
WTD	Poland	2899	http://www.ihar.edu.pl/gene_bank/
VIR	Russia	6790	http://www.vir.nw.ru/data/dbf.htm
ICARDA	Syria	6105	http://singer.grinfo.net/index.php?reqid=1151843332.3126
NGB	Sweden	2724	http://www.ngb.se/sesto/index.php?scp=ngb
JIC	UK	3194	http://www.jic.ac.uk/GERMPLAS/pisum/index.htm
USDA	USA	3710	http://www.ars-grin.gov/npgs/searchgrin.html

The development of core collections or test arrays that aim to represent a wide range of genetic variation within a restricted set of accessions with the least amount of repetition have been ongoing in a number of institutions for some years (Matthews and Ambrose, 1995; Swiêcicki et al., 2000; Coyne et al., 2005). The composition of these different initiatives varies with both the individual collection and the aims of the study. A core collection developed to represent the variation within cultivated material will differ considerably from those where the genus as a whole is the considered.

Interest in variants and mutant forms in pea has resulted in large collections of genetic stocks that is now extending to mapping populations and near isogenic lines. Early geneticists and breeders actively exchanged novel forms which over the years have coalesced into larger holdings. The first significant collection of such genetic stocks was formed by Herbert Lamprecht as part of his long career in pea genetics which spanned over 40 years (Blixt, 1963; Lamprecht, 1974). This collection was taken over by Stig Blixt, who further developed and expanded the work. He also went on to document and computerise the collection and actively promoted the use and utility of the underlying genetic information as a tool for breeding as well as research (Blixt and Williams, 1982). The collection became linked to the *Pisum* Genetics Association as the repository of seed on published mutants and their wild type counterparts. The long term future of these resources was further secured by the transfer of the active centre for this work to the John Innes Centre in the 1994. The collection has continued to develop with the same underlying aims and objectives

which is to collect, maintain and distribute genetic stocks and associated data for research, breeding and reference purposes. An online web searchable catalogue of the gene list with descriptions, images, reference germplasm and bibliography has now been developed (Ambrose, 1996; PGene).

A wide range of older heritage or heirloom material is maintained and in some cases selected by seed saver organisations (Seed Savers Exchange). These groups have sprung up in many countries and are good sources of diverse material and often have good working knowledge of their characteristics (Stickland, 2001, Irish Seed Savers Association). In addition, there are a number of good publications of detailed descriptions and illustrations of pea cultivars that offer useful reference information. As stated in section 3.1, these are often associated with one of a number of varietal classification systems current at that time (Hendrick, 1928; Mateo Box, 1955; Fourmont, 1956 and Sneddon and Squibbs, 1958). A noticeable point in comparing the cultivars described in these publications is just how wide spread the sources of this material and their general dispersal across Europe and north America.

From Mendel's seminal paper (1866), the use of pea as a model for inheritance and genetic studies has resulted in an extensive literature concerning cytology and genetics (Blixt, 1972). The somatic chromosome number of 14 was established by Cannon (1903). Studies of the pea karyotype and associated translocation points was extensively studied (Sansome, 1950; Lamm, 1951; Lamm and Miravalle, 1959; Folkesson, 1990). Lamprecht (1948) was the first author to present seven linkage groups claiming that they corresponded to the seven chromosomes of pea. The data available at the time was limited and inevitably, further work has led to extensive revisions to these original linkage groups and their chromosome assignments (Hall et al., 1997a and 1997b; Ellis and Poyser, 2002). The various genetic maps for *Pisum* are becoming increasingly well aligned as more markers are mapped and exchanged between mapping groups. The most recent map combines data from three different crosses and comprises of 239 microsatellite markers (Loridon et al., 2005) but other key maps that are of use include those of Lacou et al. (1998) and Weeden et al. (1998). A set of linkage maps that are particularly useful are three that were constructed between vining and combined peas (Ellis et al., 1992).

One of the problems still faced by breeders today is how to bridge the gap between broader genetic variation, whether in the form of exotic diversity or phenotypic variation represented in mutant collections and its availability in a form that can be easily used within breeding programs. With such a wide distributional range and long history of cultivation, the immense range of germplasm resources available for pea in *ex situ* collections represents an interesting paradox. They are considered of high value as resources which focuses around there containing important alleles and allelic combinations for the future of crop improvement, while at the same time our knowledge and understanding of the underlying structure and drivers of genetic variation remains limited (Ambrose et al., 2004). Investigations into the distribution of diversity within cultivated pea and their relationship to wilder forms have been performed for a number of reasons; to help understand and refine phylogenetic relationships within the genus, to help delineate differences between different cultivated forms (Amurrio et al., 1995) and in the structuring and management of germplasm collections. They have been used to explore the

relationships between different cultivated forms, the taxonomic structure and the organisation of germplasm collections and to assess the relationship between the different cultivated forms and wild germplasm. In recent years, the deployment of a range of molecular marker diversity studies in pea have had significant impact on the level of information that is available (Lu et al., 1996; Ellis et al., 1998; Pearse et al., 2000; Burstin et al., 2001; Vershinin, 2003; Baranger et al., 2004; Tar'an et al., 2005). The improved reliability of marker systems and the ability to develop them as high throughput systems (Flavell et al., 2003) means it is now realistic to consider the screening of whole germplasm collections. The first such example in pea is the application of retrotransposon element markers to the entire John Innes *Pisum* collection was commenced in 2000 (Flavell et al., 1998; TEGERM).

5 Major Breeding Achievements

The large range of cultivated forms available commercially by 1900 already represented a large primary cultivated gene pool. Already adapted to growing in a wide range of agroclimatic regions and with extensive variation for flowering time, plant habit and seed characters, pea breeders have had ample resources with which to work. Breeders have been consistently improving the pea crop without necessarily being able to define the genetic basis of what they have done. The majority of improvements in yield and performance of the crop have been through small incremental steps rather than large ones. While the geneticist is often a reductionist, dissecting pathways down to individual components, breeding is about the integration of a complex range of inputs and variables whose interactions are mostly poorly understood. Small wonder then that the commercial pressure of breeding results in the majority of the effort going into crosses between mostly elite material.

There are nevertheless some definable developments from recent decades that are worthy of note. 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 the planting density is such that neighbouring plants become attached to each other within the canopy, while this may keep the crop standing for some time, the canopy, in a good number of cases 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. A major contribution to combating lodging was the incorporation of the recessive allele of the *afila* gene (*af*) that converts leaflets to tendrils (Fig 2 a and b) in the 1970s leading to the development of the 'semi-leafless' pea (Snoad, 1974; Davies, 1977; Hedley and Ambrose, 1981). The presence of additional tendrils that interlock with each other help make a more rigid upper canopy, while also allowing more light and air circulation deeper into the canopy. Since the release of the first cultivars carrying this trait it has been used in breeding programs worldwide and a majority of new cultivars carry this trait. The resurfacing of an induced *afila* allele expressing an

intermediate form bearing a pair of leaflets in addition to the tendrils (Fig 2 c.) offers further possibilities for breeders to explore (Ambrose, 2004).

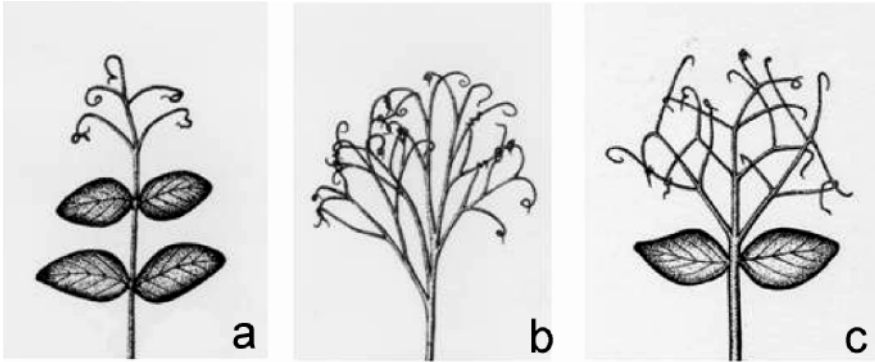


Fig. 2. Phenotypes associated with alleles of the alfalfa (*af*) locus. a. wild type *AfAf*, b. *afaf*, c. *afaf*^{1/47}.

Significant advances in the incorporation of disease resistance into modern cultivars is now becoming more routine and thus helps to reduce crop inputs. One notable example is resistance to powdery mildew (*Erysiphe pisi*) reported by Harland (1948) which still confers good resistance today and shows no sign of breaking down. The incorporation of resistance or tolerance to a range of viruses and a range of fungal diseases is becoming more standard.

The strategy with the commercial vining pea crop has been to maximise the proportion of developing embryos of desired size at the right stage of development. This has been approached using a number of strategies including selecting for high number of flowers per node (Hardwick et al., 1979), trying to develop a more determinate plant habit with a restricted the number of flowering nodes and by selecting for simultaneous flowering at multiple nodes (Marx, 1977), more ovules per pod providing more embryos at the required stage. Fasciation which result in an increase in the number of flowers borne in the apical region of the plant (Fig. 3) have been tested as an alternative means to achieving a higher proportion of embryos at the required stage of development (Gottschalk, 1977). This character exists in older long vined picking varieties and has come in and out of favour with breeders over the years as there is also a tendency in wet seasons for falling petals to become lodged in leaf axils and offer sites for botrytis and other pathogens to invade. A number of new varieties can be found described as semi-fasciated (fasciated but low to medium expression) within pea trials in the UK. While genetic variation exists for all these traits, the translation of their potential into real physiological gains within the crop has been slow. The physiological load of the developing seeds on the plants competing in the crop environment is a complex one to model (Marx, 1977) and in commercial plant breeding, the opportunities to develop different plant ideotypes to the point where they can be tested against each other is a rare event. The only trait of these that has successfully been exploited is that known as multipod where 3-4 pods are successfully held on a raceme but this type represents only a small fraction of

varieties and is not universally successful or reproducible across sites and years. It can only be hoped that opportunities to engineer further changes to the plant architecture will emerge.

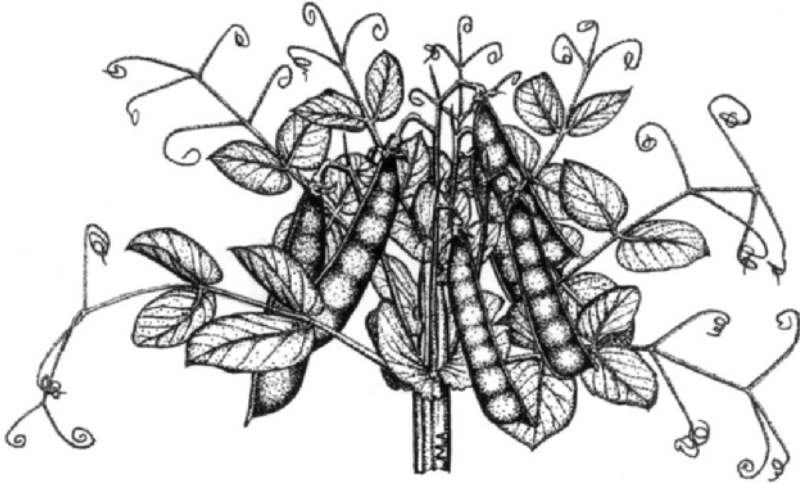


Fig. 3. Apical fasciation in cultivated pea.

6 Current Breeding Goals

The overarching goals of breeders will always be yield, quality and consistency. Dissecting these into their components traits requires a constant review of new knowledge and resources with a view to their application or incorporation into crossing programs. The ever present challenges of biotic and abiotic stresses are also a high priority for action and the changing weather patterns being experienced in many regions of the world only increases the degree of difficulty and crop management required in dealing with the crop. Having said that, breeders have consistently delivered new plant varieties that outperform earlier types. It is interesting to note however, that some older commercial lines, for whatever reason, continue to be popular and remain in cultivation. Having already developed a range of successful forms for the various market types which have come through the system, breeders can be confident that the basic plant models are fit for purpose.

In terms of plant architecture the overriding problem associated with the pea crop is still its variable standing ability. The character is frequently included in varietal assessments and the introduction of the semi-leafless form referred to in section 5.1, has certainly helped considerably, lodging remains a problem even in relatively short

strawed types. Descriptions of stiffer stemmed forms can be found in the literature but have proved disappointing. Studies into the mechanics of stem strength have not contributed anything tangible or consistent to date. Their poor description and understanding however suggests this is an area that might warrant revisiting. Fasciation and its multiple role in broadening the upper sections of the stem, synchronising flowering and clustering the pods at the top of the canopy rather than being spread throughout, is still being used by breeders who are able to select forms with moderate degree of expression hence the emerging use of the term semi-fasciated type.

The pea crop still suffers from a wide range of pests and diseases (Kraft and Kaiser, 1993; Kraft and Pflieger, 2001). Resistance or tolerance to pests and diseases, while readily taken up by some breeders are becoming more frequently used as the reliance and costs of agrochemical controls on vegetable forms becomes a more contentious issues with respect to consumers and their impact on the environment. Good sources of resistance to many pea diseases have been documented (Hagedorn, 1984; Lewis and Matthews, 1984; Ali et al., 1994). The highest priority disease targets today centre on foot and root rots, especially *Aphanomyces euteiches*, downy mildew (*Peronospora viciae*) and pea blight (a complex of species including *Ascochyta pisi*, *Mycosphaerella pinodes* var. *pinodella*, and *Phoma medicaginis*). *Aphanomyces* root rot has become one of the most destructive pea diseases worldwide. Tolerance first reported by Marx and colleagues (1972) proved unusable due to tight linkage to alleles that adversely affected the vegetable product. Kraft successfully recovered partial resistance in breeding lines with desirable horticultural traits in 1988. With the emergence of new strain and short rotations between pea crops the problem increased and with no effective fungicide treatment, efforts to find additional sources of resistance has been the subject of large-scale international collaboration to screen germplasm for new sources of resistance and incorporate sources of partial resistance into breeding programs. A number of QTL's for resistance, one that appears consistent over years and across sites and four minor ones were reported by Pilet-Nayel et al., 2002. Markers are now being developed to assist with the selection of resistant progeny for breeders. Downy mildew is present in many pea growing areas but is only of economic importance in regions which experience high temperatures and humidity and even then its severity depends on the timing of infection (Kraft and Pflieger, 1993). Resistance was reported by Matthews and Dow (1976), but recent results suggest this resistance is breaking down (Thomas et al., 1999). A renewal of effort between pathologists and breeders is therefore required to deal with this disease. *Ascochyta pisi* causes leaf and pod spot and can cause serious blemishing in vining peas. Sources of resistance and host differentials are available for all the causative species that form the *Ascochyta* complex but the complexity of the disease when encountered in the field and the multigenic nature has lead to slow progress in the utilisation of the available sources of biological resistance. High priority target pests of pea include aphids (*Acyrtosiphon pisum*) and bruchids (*Bruchus pisorum*, *B. affinis*). Large infestations with aphids can cause stunting of the plant and damage to foliage and pods by their feeding. These often occur as temperatures rise and there is a concomitant problem with drought stress under which the symptoms may become even more severe. They also act as a vector in the transmission of some 30

plant viruses. Bruchids are a serious problem in growing regions where they occur feeding on pollen and then go on to cause damage to the developing seed where the larvae feed and continue their development thus rendering the seed unmarketable. There are no reports of resistance to bruchids in cultivated sativum germplasm although resistance has been reported in studies from the secondary gene pool of *Pisum fulvum* (Hardie, 1990; Clement et al., 2002). Recent work has also established an inducible resistance conditioned by the recessive allele for neoplastic pods (*np*), where neoplastic outgrowths of undifferentiated tumour-like cells develop in response to oviposition on developing pods which impede larval entry into the pod (Doss et al., 2000). The possibility of combining these findings offers further potential advances in increasing the plant defences against this pest.

Quality traits and post harvest changes represent the final category of challenges to breeders. Having developed material that germinates, grows and survives to be harvested, maintaining the quality of the product to the consumer poses technical as well as biological problems if premium prices are to be achieved. One way of approaching this is to seek to minimise the wastage of what is harvested by seeking more uniformity of the developmental stage of the seed or pods, or by seeking small incremental steps that can be achieved by incorporating traits resulting in peas retaining a darker colour and not becoming bleached. At present, little is known of the complex metabolite profile of what makes a good vegetable pea and which compounds cause problems. New techniques to explore these questions are now being tested but currently, these complex issues are poorly understood. The produce delivered by breeders and growers across the market types is already a good one and any progress made is more likely to be as a result of closer cooperation and operational management of the supply chain.

7 Breeding Methods and Techniques

Pea behaves and is managed as an inbreeding crop. There is a low rate of outcrossing estimated at below 1% (Gritton, 1980) although this figure may rise under stressed conditions where flowers are generally smaller and the stigmatic surface protrudes through the keel. As an inbreeder, the most widely practiced breeding method is the pedigree breeding system through transgressive segregation from crosses. Selection for high heritability traits is frequently practised in the early generations before lines are grown out as small plots around the F_4 generation. Some breeders employ the strategy of growing and selecting alternate generations (F_3 and F_5) at off-site locations in countries in the opposite hemisphere immediately following harvest in their target region to reduce generation time. There is widespread use of the single seed descent system utilising glasshouses or plant growth rooms to speed up early generations while also maintaining a wider level of variability between lines before growing out small micro-plots of plant progenies for field evaluation and selection. Bulk selection is also used by some breeders where the F_2 is grown as a bulk which is split into smaller units, a portion of which are grown in small plots while others are grown as individual plants to facilitate selection based on plot performance. Recurrent backcross and selection is commonly used to introduce single desired trait

such as disease resistance or quality trait from less adapted material into elite backgrounds. An example of this is the development of lines carrying resistance to pea seed-borne mosaic virus (PsBMV) in eight varietal backgrounds (Muehlbauer, 1983).

There is a long history of mutation breeding in pea where chemical or radioactive mutagenic agents are deployed on commercially proven cultivars of the day to induce random mutations across the genome some of which may prove useful in crop improvement such as stem thickness or simultaneous flowering but many more may be of potential use in breeding (Blixt et al., 1991). The method was widely adopted in the 1960's and 70's in Sweden (Lamprecht, 1974), Italy (Saccardo et al., 1986), Germany (Gottschalk, 1977; Gottschalk and Wolff, 1977), Poland (Jaranowski and Mickle, 1985) and Bulgaria (Vassiliva, 1978). All are examples of collaborations between research geneticists and breeding programs, mostly in the public sector. The method has proved of mixed fortunes in that it has undoubtedly resulted in many novel alleles at both known and new loci which have contributed very significantly to research into the understanding of basic plant development and to the pool of variation that is available for breeding, while in practical breeding terms, few induced mutations have found their way into finished varieties to date. The first registered variety was of Stral-árt in 1954 (Gelin, 1955) with a few others following in the late 1970's to mid 1980's (Jaranowski and Mickle, 1985; Saccardo et al., 1985). None have been reported in the intervening period. In some respects this is a rather harsh criticism, as induced mutations should be taken in a more collective context of the use of mutations in the broad sense. On this basis it is clear that mutants have provided a significant range of variation that is widely represented in modern breeding material. The frequency of uptake of any single trait into breeding is extremely low in both forms but they do occur. It is important therefore that they remain available and re-evaluated over time to ensure earlier efforts of inclusion do not prejudice future gains. Each generation of breeders and researchers needs to become acquainted with this wider variation as they are represent some of our most readily available resources for crop development. A wide range of mutations isolated from past programs, have been incorporated into public germplasm collections, the majority are still available and studied today (Blixt, 1972; Swiêcicki et al., 1981; PGene). Each generation of breeders and researchers needs to become acquainted with this wider variation as they are represent some of our most readily available resources for crop development.

While there are no examples of mutation breeding being undertaken today, there are numerous mutagenesis programs to be found in public sector research. These programme underpin basic research and resource development in plant developmental genetics and genomics. The regular dialogue and collaboration between the research and breeding communities means that any new allelic variation of potential agronomic use are generally readily available to breeders and joint development of material is also not uncommon (GLIP). Recent examples of induced chemical mutations of interest to breeders include new genes regulating basal branching (Rameau et al., 1998) and bulbous base (*blb*) which results in a thickening of the hypocotyl region at the base of the stem (Kosterin and Rozov, 1993) which may help in strengthening the stem base which is naturally weak. Alleles at the *rug3* locus

encoding the enzyme plastidial phosphoglucomutase which is involved in the starch biosynthetic pathway, have resulted in a patented method for increasing sucrose content of plants (Harrison et al., 2000). This gene is of potential value in breeding for the frozen pea market as its regulation results not only elevated levels of sucrose compared to conventional wrinkled high sucrose forms, but sucrose levels remain elevated over a longer period of seed development thus offering an extended harvesting window for the crop (HU9903062 1999).

Apart from isolated instances of close linkage between morphological characters, The identification of linked markers to genes of interest for use in marker assisted selection (MAS) within breeding programs started with the mapping of isozyme markers (Weeden and Marx, 1984). The prime targets for MAS are traits that express late in plant development. This allows for the early screening of large populations of seedlings, which would be costly to grow on to screen later on. Such targets include disease and lodging resistance and seed characters. The first isozyme marker linked to a resistance gene was that of alcohol dehydrogenase (*Adh-1*) linked to resistance to pea enation mosaic virus (*En*) and was reported by Weeden and Provvidenti (1987). The rapid developments in marker systems over recent years, their increasing ease of application and lower unit costs has resulted in breeders becoming more interested in deploying MAS as they become available. Two recent examples related to disease resistance are the development of PCR markers designed from cDNA-AFLP fragments providing tight linkage to genes (*sbm-1*, *mo*) conferring resistance to pea seed borne mosaic virus (Gao et al., 2004) and an SSR marker suitable for MAS for powdery mildew resistance in pea (Ek et al., 2005). The recent development of a simple marker system developed to enable the identification and selection of progeny with low levels of seed trypsin inhibitors is an example of the type of resources that are increasing as a result of the more detailed genetic maps (Page et al., 2003). A further example that highlights a future development involves the identification of quantitative trait loci (QTLs) for lodging resistance using two markers that successfully led to the identification of F2:3 families with significantly lower lodging scores (Tar'an et al., 2003; Warkentin et al., 2004).

8 Integration of New Biotechnologies in Breeding Programs

The use of new technologies in breeding vegetables peas is restricted to the frozen and canning sectors which are the only sectors that generate sufficient revenue to invest in such operations and even here, the R&D spends are not large. As stated previously, there are a number of examples of vegetable and combined pea breeding both employ marker assisted selection and mapping capacity although this number is still limited. This section presents examples of other technologies that have or are still being used in breeding programs but the individual efforts remain focused and relatively small. It is true to say that the combined commercial breeding sector in pea is insufficient to sustain the development of such technologies itself and relies on public sector and consortium arrangements to help develop and sustain innovation in these areas. Their contribution is often by way of material, expertise and other in kind contributions. The main regulators that restrict the uptake of new technologies

in breeding programs are consistency and cost per data point or sample. As reproducibility both within and across groups and platforms improves and the cost per assay or run comes down, the more likely it is that the utilisation of such technologies alongside breeding programs will become more widespread.

Transformation and regeneration protocols have been available for pea for some time and are regularly used in research work. The most widely adopted method for pea is that of *Agrobacterium tumefaciens* mediated transformation (Puonti-Kaerlas et al., 1990; Schroeder et al., 1993; Bean et al., 1997). Difficulties arise from the fact that only a small proportion of cultivars prove suitable for either regeneration or transformation and the basis of this variability is not understood. Having said that, a sufficient range of cultivars have been found suitable for the work to not be unduly limited by this factor. Somoclonal variation arising from the regeneration of plants from callus led to the use of cotyledonary meristems from freshly imbibed seed as a source of tissue for transformation (Bean et al., 1997). Improved strains of *Agrobacterium* have only resulted in slight improvements in performance and overall transformation efficiencies are still only around 4% at best. Nevertheless, the use of these procedures in research ensures that capacity in this field is being maintained. The use of these technologies in breeding is limited to proof of concept. One example is the partial resistance to alfalfa mosaic virus (AMV) gained as a result of transformation with a chimeric virus coat protein gene (Grant et al., 1998). A second example is the transfer of α -amylase inhibitor (α -A1) and the promoter phytohemagglutinin, both found in *Phaseolus vulgaris* and were shown, when constitutively expressed in pea, to confer resistance to pea weevil (*Bruchus pisorum*) (Schroeder et al., 1995). The expression of the inhibitor served to block the development of the larvae at an early stage in development and so seed damage was minimal and seed quality much improved. Legal complications over some of the technology used effectively stopped this work at the early stage of field testing. The transfer of herbicide resistance both as a reportable marker and a trait to benefit the crop have been reported but not carried through to commercial release. While GM crops are on the increase in many parts of the world, the adverse reaction to GM crops in Europe and the low rates of transformation and target genes to transfer have all contributed to the pea breeding industry not engaging in the development of GM peas to date. In fact, in contrast to soya, peas can be frequently found to be promoted using their non GM status as a positive marketing strategy. No doubt this situation will change over time as further refinements to the technologies and good candidates for gene transfer emerge to produce real benefits for the grower and consumer. A recent improvement to the process has been the development of so called 'clean gene technology' whereby vectors are formed that carry two T-DNAs, the first carries the gene of interest and the second the selectable marker. In some cases the two genes insert into unlinked locations and thus plants containing only the gene of interest can be identified among the progeny.

The every growing array of technologies and genomic tools that are being developed for model species are already starting to impact on breeding of a number of crop species. For pea the model legume species are *Lotus japonicus* and *Medicago truncatula*. The developments include platform technologies and resources that bridge the gap between functional and structural genomics and link their discovery in

models to application in crops (Waugh et al., 2006). Genotype assisted breeding has recently emerged as an approach to marshalling functional markers and informatics that in principle will allow *in silico* design and selection. A big challenge that these approaches present is the requirement for detailed phenotypic data of agronomic and processing traits. While the costs remain relatively high for inbreeding species and especially where they represent only a minor crop, these will come down in time and MAS and selection will gradually evolve into genomics assisted breeding (Varshney et al., 2005). A clear mobilisation of the legume community to address these concerns and develop a combined efforts between researchers and breeders is reflected in a number of ongoing multidisciplinary programs. One example is the pulse crop improvement network (PCGIN) in the UK which brings together legume research community, breeders and processors where correlating genotype to phenotype is one of the prime objectives along with the development of germplasm and genomic resources for use in the applied sectors. The development of forward and reverse genetic platforms are further examples of new tools that are of potential benefit to breeders. Two complementary reverse genetics tools developed as part of the European grain legume integrated programme (GLIP) are now operational. The first is a pea tilling platform (PETILL) based on two EMS mutagenised populations and the rapid systematic identification of mutations in target sequences. The second is allele mining or EcoTILLING which is based on the analysis of natural allelic variation for the gene of interest in sets of germplasm. Both are high throughput systems that represent an effective way to generate or identify variant alleles in specific genes of interest.

The rapid developments in comparative mapping and sequencing across legume species is set to greatly benefit pea breeding through access to a wide range of tools and resources. The high degree of co-linearity between the *Pisum* and *Medicago truncatula* (Kalo et al., 2004), genetic maps and the large international sequencing effort in *Medicago* and *Lotus japonicus* brings an unprecedented range of tools, resources and data into the compass of those researching and breeding pea.

One of the current priority areas for legume research is in exploring the complex interactions with micro-organisms, particularly, arbuscular micorrhiza and nitrogen fixing bacteria. One of the primary features that pea, along with many other legume species exhibit, is their ability to fix atmospheric nitrogen through the complex and elegant process of symbiotic nitrogen fixation in association with *Rhizobium leguminosarum* (Brewin et al., 1993). This process enables peas to grow well on poorer soils and agronomic manuals state no N-fertilizer is required. Exploration of the efficiency of plant genotypes, bacterial strain and their interaction have highlighted significant effects and highly heritable variation (Hobbs and Mahon, 1982; Skøt, 1983). The understanding and manipulation of these processes has been a key area of research over many decades. The *sym2* gene associated with race specificity identified in peas from Afghanistan (Lie, 1978) and subsequently found to be widespread in ecotypes and landraces from the eastern mediterranean, Turkey and Iran (Young and Matthews, 1982), opened up the possibility of linking this specificity to 'improved' strains of *Rhizobium* that could be applied as inoculum to the crop in an effort to improve the nitrogen fixation capacity of the crop. The inability of the introduced strains to compete with the natural *Rhizobium* strains led