

Contents

PGA Notes	ii
Research Papers	
The <i>Coch</i> gene controls the subsequent differentiation of pea axial meristems into lateral structures Rozov, S.M., Voroshilova, V.A., Tsyganov, V.E., Priefer, U.B., Borisov, A.Y. and Tikhonovish, I.A.....	5
The new xantha-variegata-viredescens mutation in pea — <i>xavs</i> Rozov, S.M.	11
A new chlorophyll gene (<i>xach</i> , <i>xantha chlorescens</i>) in <i>Pisum sativum</i> L. located on LG II Święcicki, W.K., Gawłowska, M. and Nawrot, Cz.	14
Use of Supernodulating Mutants in Pea Breeding Sidorova, K.K.	17
In situ evaluation of a <i>Pisum sativum</i> subsp. <i>elatius</i> population from the valley of the river Pčinja in southeast Serbia Ćupina, B., Zlatković, B., Smýkal, P., Mikić, A., Jajić, I., Zeremski-Škorić, T. and Medović, A.	20
Some agronomic aspects of the intercrops of semi-leafless and normal-leafed dry pea cultivars Antanasović, S., Mikić, A., Ćupina, B., Krstić, Đ., Mihailović, V., Erić, P. and Milošević, B.	25
Forage yields in the intercrops of pea with other cool season annual legumes Krstić, Đ., Mikić, A., Ćupina, B., Antanasović, S., Mihailović, V., Erić, P. and Pejić, B.	29
Intercropping pea with eight cereals for forage production Mihailović, V., Mikić, A., Kobiljski, B., Ćupina, B., Antanasović, S., Krstić, Đ. and Katanski, S.	33
Can we reconstruct the most ancient words for pea (<i>Pisum sativum</i>)? Mikić, A.	36
Brief Communications	
Registration of JI 3564 'Karl's Longpod' a Heritage Pea variety into the John Innes <i>Pisum</i> Collection Mike Ambrose	43
Abstracts and Short Papers Presented at the NAPIA Meeting, San Juan, Puerto Rico	45
Membership List	67

Pisum Genetics Association Notes

The Pisum Genetics Association (PGA) is a non-profit, unincorporated organization established to foster genetic study of the pea, to facilitate the exchange of information and to ensure preservation of valuable genetic stocks. The journal, **Pisum Genetics**, is the principal means of meeting these aims. Published annually in one issue, Pisum Genetics contains reports of research findings and other information of interest to researchers studying the genus *Pisum*.

Membership and Dues

Membership in the PGA is open to all interested persons and organizations. Dues are US \$15.00 per year. These dues are used to help defray the costs of publishing and mailing Pisum Genetics. Members are requested to send checks or bank drafts in US currency only. Such efforts avoid bank charges, which can exceed \$10.00 per check in some currencies. Please make checks payable to Pisum Genetics Association and send to Kevin McPhee, NDSU Dept 7670, P.O. Box 6050, 370G Loftsgard, Fargo, ND, USA, kevin.mcphee@ndsu.edu. Pisum Genetics will be sent upon receipt of dues.

All members of the PGA are welcome to submit manuscripts on topics appropriate for Pisum Genetics. The deadline for such submittals is usually September 1 of the year of publication, allowing for sufficient time for review and revision of manuscripts. The journal provides opportunities for both refereed and non-refereed manuscripts, publication is within 3 to 6 months of submittal, and there are no page charges. Pisum Genetics is indexed in CAB International. As has been the case for the last decade, a web-based version is available shortly after publication of the hard copy (<http://hermes.bionet.nsc.ru/pg/>).

Submission of Manuscripts

When submitting an electronic copy of a manuscript, please use Microsoft Word when possible. Other formats can be handled, but these will usually be converted to Word for processing. It is also helpful if authors avoid the use of extra formatting in their submissions (headers, sections, automatic indentation) as these generally have to be eliminated when preparing the manuscript for final printing. Tables created in Word using the table function are most likely to survive the formatting process intact. We have severe limitations on support staff, and the removal of interfering formatting can occasionally be quite time-consuming. Graphics reproduce best at 300 dpi.

Format of Manuscripts

Manuscripts are generally separated into two types, research papers and brief communications. Manuscripts submitted as research papers will be subjected to peer review. Research papers should be written concisely, with a short introduction presenting the purpose for the study, a materials and methods section with essential details and references to techniques, a results section and discussion section interpreting the results and integrating findings with those of other workers. Section headings are not obligatory. The length of manuscripts is flexible. Manuscripts under five journal pages are encouraged. Longer papers will be considered on their merits and space availability in the volume. All research papers must be treated similarly to submissions to other refereed journals in that they should contain original work not currently submitted to any other journal.

**Brief
Communications**

Brief communications are to encourage a rapid sharing of new results that may not meet the standards of a research paper (i.e. allelism tests not completed, map locations only approximate). Brief communications can also be used to describe the availability of new germplasm. Brief communications will not be subjected to peer review although the editor reserves the right to screen papers for appropriateness and to review submissions for clarity and brevity. Such submissions will be limited to approximately one page and should be narrowly focused. Otherwise, the format of brief communications (tables, references, etc.) should correspond to that of a research paper. Publication of a brief communication in Pisum Genetics does not preclude publication of a full paper on the same subject in a later issue or in another journal.

Member News

Dr. Serge Rozov continues to serve as the webmaster for the PGA website (<http://hermes.bionet.nsc.ru/pg/>) and has extended the coverage of previous issues available on the site back to volume 11 (1979). Electronic versions of volumes 9 and 10 are expected to be available on this site shortly. Hard copies of volumes 10 through 43, plus a selected number of earlier volumes can be obtained by contacting the editorial office.

Financial Report

The PGA continues to be fiscally healthy. Income from dues and subscriptions has been adequate to cover expenditures. We have adequate funds in savings/certificate of deposit accounts that can cover most PGA expenses for several years. The financial accounts were transferred to Fargo, North Dakota, in late 2008 and are expected to be maintained there for the foreseeable future. The financial report is based on the current status of funds in Fargo, North Dakota. An accounting error was recognized in 2011 and a corrected financial statement for 2010 is included in Volume 43. Expenditures for distribution of Volume 42 and publication and distribution of Volume 43 are not included in this report.

Financial Report for Volume 43

Account balances from preceding year			
	Checking (Nov. 2010)	658.41	
	Savings/Certificate of Deposit	6502.45	
	Total assets		7160.86
Income			
	Subscriptions	345.00	
	Bank interest	5.96	
	Total income		350.96
Expenditures			
	Printing costs (volume 42)	602.89	
	Total expenditures		(602.89)
Account balances			
	Savings/CD (Oct. 2011)	6508.41	
	Checking (Sept. 2011)	400.52	
	Net balance for Pisum Genetics Association		6908.93

K.E. McPhee for the Coordinating Committee:

M. Ambrose	K.E. McPhee	S.M. Rozov
T.H.N. Ellis	I.C. Murfet	W.K. Święcicki
O.E. Kosterin	J.B. Reid	N.F. Weeden

Financial Report for Volume 42, Corrected

Account balances from preceding year			
	Checking	1577.85	
	Savings/Certificate of Deposit	6496.49	
	Total assets		8074.34
Income			
	Subscriptions	465.00	
	Bank interest	5.96	
	Total income		470.96
Expenditures			
	Printing costs (volume 41)	870.26	
	Mailing expenses (volume 41)	514.18	
	Total expenditures		(1384.44)
Account balances			
	Savings/CD	6502.45	
	Checking	658.41	
Net balance for Pisum Genetics Association			7160.86

The *Coch* gene controls the subsequent differentiation of pea axial meristems into lateral structures

Rozov, S.M.¹,

Voroshilova, V.A.²,

Tsyganov, V.E.²,

Priefer, U.B.³,

Borisov, A.Y.² and

Tikhonovish, I.A.²

¹Institute of Cytology and Genetics SD RAS, Novosibirsk, Russia

²All-Russia Res. Institute for Agricultural Microbiology, St. Petersburg, Russia

³Institute of Biology I, RWTH Aachen University, Aachen, Germany

The *coch* mutant is well known from Wellensiek (1) and Marx (2) initial studies and for many years it was considered to affect only stipules and flowers of pea plants. The *coch* phenotype is characterized with strongly reduced, sometimes filimorpha stipules. In some cases, particularly in the middle nodes of the plant, stipules can transform into small additional, adventitious leaves with leaflets and tendrils. The flowers of the *coch* mutants have numerous abnormalities, such as additional vexillum and wings instead of keel petals, an irregular number of stamens and low fertility due to abnormal opening of flowers (1, 2, 3).

In the last ten years it has been established that the compound leaf of pea forms as a result of interactions within a pool of homeiotic genes including *Uni*, *Coch*, *Tl* and *Af*. Interactions of genes *Uni* and *Coch* determine stipule formation, whereas interactions of *Uni*, *Tl*, and *Af* determine the formation of leaflets and tendrils (4). In addition, *Coch* is strongly involved in flower morphogenesis (unlike genes *Tl* and *Af*), affecting the shape and number of its elements (4, 5). All these data indicate that *Coch* could be one of the key factors in the formation of meristems of numerous organs and tissues of the plant, maybe not only pea, but in other Fabaceae as well. In addition, we found that *coch* had strictly visible effects on the pea root nodule meristem development and differentiation (6). The effects of the *coch* mutation on root nodule development have been confirmed by Ferguson and Reid (7).

In earlier research we found three new EMS and γ -ray induced mutants. Two of them showed a phenotype similar to *coch* — SGE239 (EMS) and SGR856 (γ -ray) (6,8,9), and one displayed a phenotype resembling *het* (heterophyllus) — SGE624 (9). The SGE624 mutant phenotype was characterized by stipules that were less strongly narrowed than in *coch*, but varied in shape and nearly normal flower formation. A number of allelic tests performed between these mutants and classic *coch*-lines NGB1743 (Nordic Gene Bank) and Wt11745, Wt11303 (Wiatrowo) revealed that all three new mutants are alleles of *coch* (9). SGE624 appeared to carry a weak *coch* allele — *coch*^{het} (9).

Root systems of the above mutants and standard *coch* lines were in general visually normal. All mutants formed active nitrogen-fixing nodules evidenced by pink coloration due to leghemoglobin synthesis. In contrast to the wild-type, small well-developed roots were formed at the tips of the mutant nodules. Only SGE624 (*coch*^{het}) did not produce this root-like structure, or produced only a few of them.

EMS-induced *coch* mutant line SGE239 was selected for further study because it had relatively good fertility and decreased plant size. A portion of the root system and an individual nodule of both SGE239 and ancestor line SGE is shown in Figure 1. It seems clear, that the *coch* mutant nodules produce additional rootlets. These rootlets develop from the nodular lateral meristem that forms a heavy root-tissue layer around the nodule. It was presumed that these abnormal nodules were able to fix nitrogen normally because the mutant plants did not show any signs of nitrogen starvation.

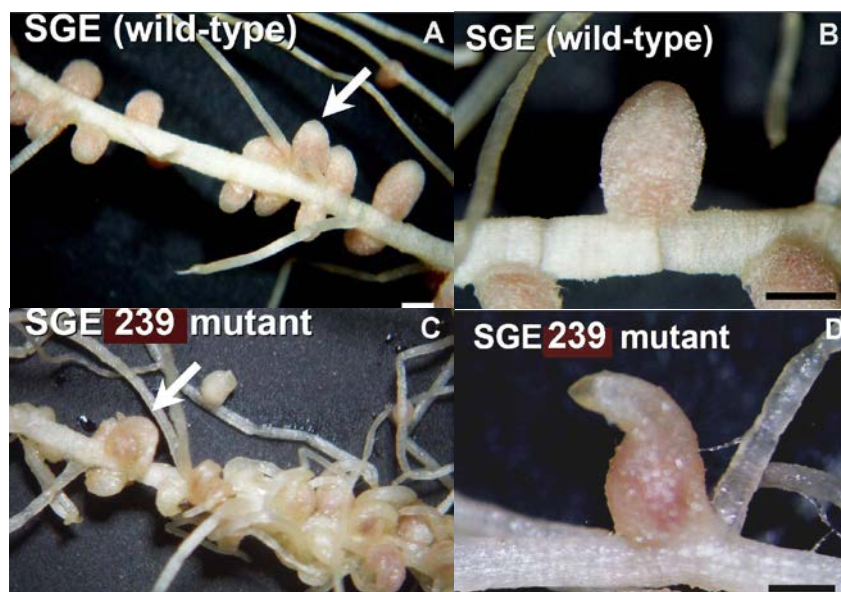


Figure 1. The root nitrogen-fixing nodules of mutant coch line SGE239 (C, D) and SGE ancestor line (A, B).

The stage at which nodules in the SGE239 mutant were transformed to a rootlet was identified by microscopic analysis. It was found that early stages of root nodule development in the mutant were similar to the parental line SGE.

Both wild type and mutant plants show the normal process of root hair curling, infection thread growth, nodule induction and primordium formation. Then similarly in both lines the infection thread penetrates nodule primordium, bacteria endocytosed into plant cell cytoplasm and immature nodular circular meristem is differentiated at the distal end of the nodule primordium. The meristem of mature indeterminate nodules is formed from one part of the circular meristem at the next stage of wild-type nodule development. But at the same time in SGE239 nodules a root meristem is developed from the part of nodular immature circle meristem at the distal end of young nodules. At a later stage, root growth in the mutant is observed at the tip of the nodule and the vascular system of this rootlet is connected to one of the vascular bundles of the nodule (Fig. 2). Thus, the pea gene *Coch* acts at the stage of mature indeterminate nodule meristem development, specifying nodule or root meristem identity.

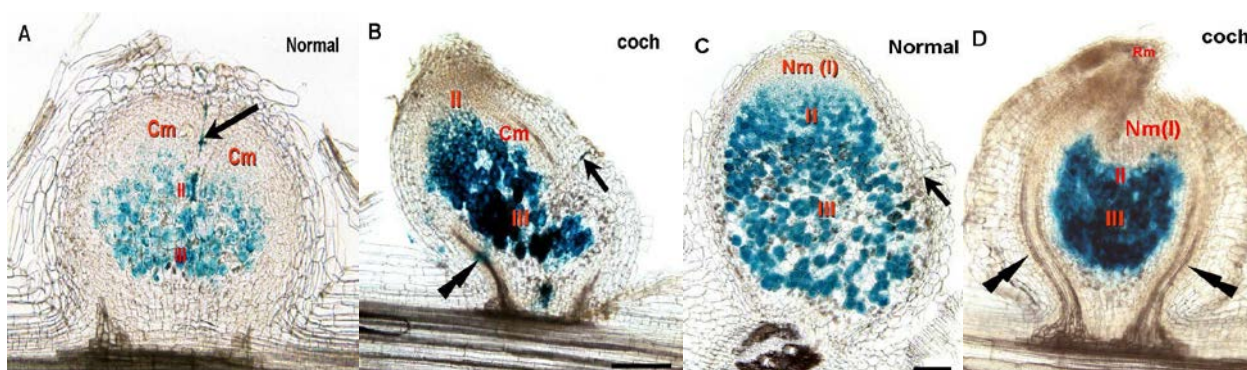


Figure 2. Two late stages of pea root nodule formation. A, C — ancestor line SGE; B, D — coch mutant line SGE239. Arrows indicate the infection threads; Double arrows specify the forming root vascular system. Cm — immature circular nodular meristem; Nm (I) — mature nodular meristem; II — infection (endocytosis) zone; III — nitrogen-fixation zone; Rm — rootlet meristem.

SGE239 mutant plants formed elliptical or spatulate stipules (Fig. 3A). These stipules resembled simple leaves with petiole and one leaf blade. Mismatched stipule pairs were also observed. For example, stipules of the same pair could be different in size or only one stipule was developed. Nevertheless, it is well known that some mutant alleles of *coch* can transform stipules into additional lateral compound leaves (1, 2, 4), as shown on Fig. 3C (4).

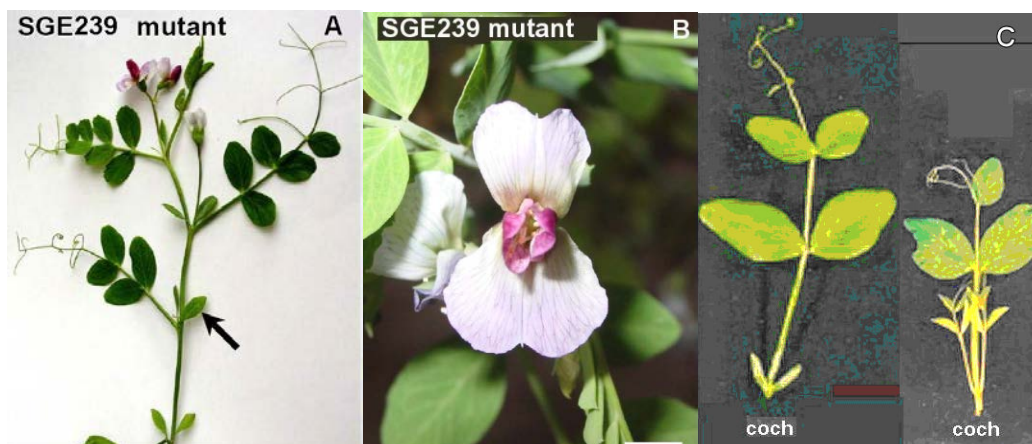


Figure 3. Stipules and flower of *coch* mutants. **A** — SGE239 plant. Arrow indicates reduced stipules. **B** — Double-vexillum opened flower of line SGE239. **C** — Pea compound leaves with *coch* variegated phenotype: with reduced stipules (left) and additionally formed lateral compound leaves (right) (figure from Yaxley et al. 2001 (4)).

SGE239 mutant flowers are open and the pistil and stamens are not hidden by the two fused keel petals which are free and frequently transformed into extra vexillum or wing structures. The general number of petals is often increased and they are of variable shape. The double-vexillum opened flower of SGE-239 is shown on Figure 3B. Stamen number is also frequently increased and their bases are not fused into a tube. Usually one carpel is observed in mutant flowers but bicarpellum flowers were also found. Flowers are partially sterile due to the opened-flower type and mutant plants have more lateral branches in comparison with the wild type.

Thus, it was found that development of stipules, flowers and nodules are impaired in the SGE239 mutant line which is typical for all *coch* mutants.

According to Gourlay et al. (10), *Coch* acts in the course of compound pea leaf development at the stage of stipule meristem formation determining its fate to be a stipule meristem. This apparently gives evidence of a common mechanism of the *Coch* gene action to determine the fate of meristems during formation of various pea organs.

The current model of the formation of compound pea leaf (11) considers the gene *Uni* as a key gene in determination of meristems of lateral structures of a compound leaf. According to this model *Uni*, alone or in interaction with other genetic factors, “tries” to transform any indeterminate meristem of the leaf primordium into the rachis meristem, i.e. converting the leaflet meristem to the one producing a shoot-like structure. Expression of the genes *Tl* and *Af* in the corresponding lateral meristems of the leaf leads to inhibition of *Uni* action and determines their fate to be meristems of leaflets and tendrils. *Coch* also inhibits the effect of *Uni* at the base of the leaf that leads to the isolation of the future stipule meristems from partly undifferentiated cells of rachis meristems and to its differentiation into the stipule meristem.

In *coch* mutants a prolonged expression of *Uni* in the stipule primordia leads to the situation where the stipule meristem does not differentiate at a time and retains its original predetermination to be a leaf

rachis. As a result, depending on the duration of elevated expression of *Uni*, either a simple leaf (short action) or a lateral compound leaf (long action) are formed instead of a stipule. Thus, the *coch* mutation deprives the corresponding meristem of the ability to further differentiate and its development progresses toward basic axial differentiation.

During compound leaf formation in *coch* mutants the lateral meristem at the base of future leaves loses the ability to differentiate into stipules, but maintains basic differentiation of the compound leaf rachis, and as a result an adventitious lateral compound leaf is formed (Fig. 4).

We do not know what meristematic factor is inhibited by *Coch* during the flower corolla and root nodule

formation, but taking into account that the meristem-determination mechanism is highly conserved (12), we may conclude that if this is not *Uni*, it is a close homolog. Obviously, one of the external circles of the flower, the corolla, can be considered as a separate shoot. If the lateral meristem located at the base of the axis of this shoot loses its ability to differentiate into the keel petals in *coch* mutants it differentiates in a basic shoot-corolla way and as a result the *coch* plants fail to develop true keel petals, but have additional slightly differentiated wing-like petals and sometimes a second additional vexillum (Fig. 4).

During symbiotic root nodule formation in *coch* mutants the lateral part of its circular meristem apparently loses the ability to differentiate into nodule tissues leading to its reversion to root type. As a result, the lateral (cortical) part of the nodule gets thicker, total nitrogen fixation zone is reduced and a part of nodule meristem produces roots instead of nodule tissues (Fig. 4).

It is assumed that mechanisms of formation of various plant meristems are highly conserved (12). The genes controlling meristem formation and functioning could be recruited in evolution to control pea compound leaf and root nodule meristem formation and functioning. Therefore, genetic studies of legume compound leaf and nodule formation can be important not only for understanding the morphogenesis of organs specific to the Fabaceae family, but also for understanding the basic mechanisms of plant development.

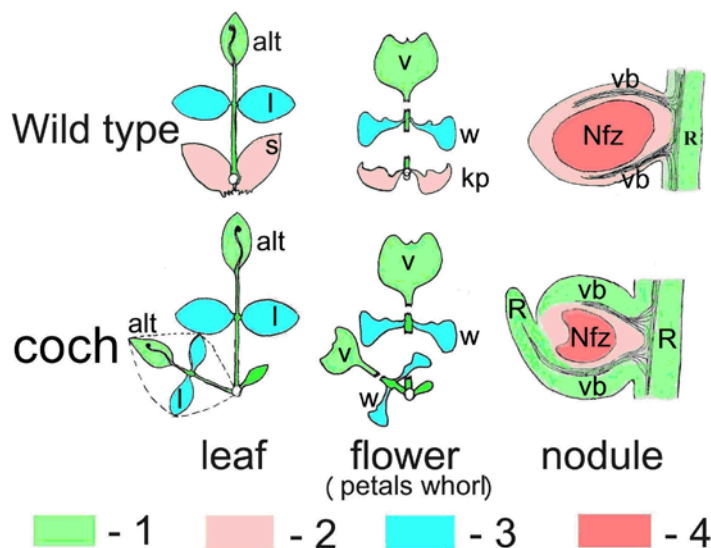


Figure 4. Effects of mutation in the *Coch* locus on various lateral meristem determination. 1 — organs with basic axial *Coch*-independent meristem determination; 2 — lateral organs with *Coch* meristem determination; 3 — lateral organs with meristem determination by other homeiotic genes; 4 — nodule nitrogen-fixation zone. alt — apical leaflet of tendril; l — leaflet; s — stipule; v — vexillum; w — wings; kp — keel petal; R — rootlet; vb — vascular bundles; Nfz — nitrogen-fixation zone.

References

1. Wellensiek, S.J. 1962. *Genetica* (Netherlands) 33:145-153.
2. Marx, G.A. 1969. *Pisum News Letter* 1:20-21.
3. Blixt, S. 1972. *Agri Hort. Genet.* 30:1-293.
4. Yaxley J. L., Jablonski W. and Reid J. B. 2001. *Ann. Bot.* 88:225-234.
5. Kumar S., Sharma V., Chaudhary S., Kumari R., Kumari N. and Mishra P. 2011. *J. of Genet.* 90:309-314.
6. Voroshilova V.A., Tsyganov V.E., Rozov S.M., Priefer U.B., Borisov A.Y. and Tikhonovich, I.A. 2004. In: *Biology of Plant-Microbe Interactions*, 4, *Molecular Plant-Microbe Interactions: New Bridges Between Past and Future*. Edited by Tikhonovich, I., Lugtenberg, B. and Provorov, N. pp. 376-379. International Society for Molecular Plant-Microbe Interactions, St Paul, MN, USA.
7. Ferguson B.J. and Reid J. 2005. *Plant Cell Physiol.* 46:1583-1589.
8. Gorel F.L., Rozov S.M. and Berdnikov V.A. *Pisum Genetics* 30:9-11.
9. Rozov S.M., Gorel F.L. and Berdnikov V.A. *Pisum Genetics* 24:82.
10. Gourlay C.W., Hofer J.M.I. and Ellis T.H.N. 2000. *The Plant Cell* 12:1279-1294.
11. Hofer J.M.I. and Ellis T.H.N. 1998. *Trends in Plant Science* 3: 439-444.
12. Ezhova T.A. 2007. *Russian Journal of Developmental Biology* 38:363-373.

The new *xantha-variegata-viredescens* mutation in pea — *xavs*

Rozov, S.M.

Institute of Cytology and Genetics SD RAS Novosibirsk, Russia

Leaf chlorophyll variegation is occasionally observed in many plants. Usually it looks like white, yellow or dark green spots or irregular regions on leaves. Mutations in both nuclear and organelle genomes reportedly may cause this leaf variegation (1). It raises a question why and how two neighboring sectors containing different cell types, a green cell with normal-appearing chloroplasts and a white cell with abnormal plastids, can be formed in one leaf tissue when the genome of all cells is identical. The precise mechanism leading to such pseudochimeric chloroplast development in the same leaf tissues remains poorly understood. One possible explanation for generation of non-identical variegated sectors in each leaf is a threshold level of factors that arrest proplastid differentiation into chloroplasts (2).

Variegated-type mutants are more rarely observed in plants than the ordinary chlorophyll mutants. In *Arabidopsis thaliana*, only four loci are known to cause the variegated phenotype (3). During experimental mutagenesis, pea mutants with *variegata* or *maculata* phenotypes appear two orders of magnitude less frequently than the other chlorophyll mutants (4, 5). Only four to six pea variegated mutants have been previously reported and almost all of them appeared to be extinct (6). For the above reasons, isolation of a new pea *variegata* mutant is of great interest in the course of studying the mechanisms of variegated plant appearance and chloroplast differentiation and survival.

Screening M₂ progeny of the EMS-treated pea line SGE revealed a new mutant SGE0802 characterized with the strong *xantha* effect on shoots and leaflets of the first expanded leaf (Figure 1). These structures are brilliant yellow, without any green spots even grown in the sunlight. Leaflets at the next three nodes (2-4) of SGE0802 sequentially and progressively become green — yellow sectors become pale or even white and green sectors arise in geometric progression from one node to the next (Figure 2). The 5th node of the mutant plants becomes completely green without any allusion of yellow or other colored sectors. It is notable, that among nearly 5000 plants analyzed, none become fully green at the 4th or 6th node — it was always the 5th node in all cases. The upper part of the SGE0802 plant is completely normal and fertility is not affected.

To determine the inheritance type of SGE0802 mutation, we crossed it with the parental line SGE in both directions, using the mutant as either the male or female



Figure 1. SGE0802 (*xavs*) seedling (A) and progressive greenish gradient along seedling (B). Digits 1–5 indicate number of node.



Figure 2. The 3^d node of a SGE0802 (*xavs*) seedling. Pale yellow, green and dark green sectors are visible.

parent. In both cases, all F_1 progeny were normal, without any variegation on leaves and all shoots were green. Segregation results in the F_2 for these crosses are shown in Table 1. In both crosses, the ratio of normal and variegated seedlings segregated did not differ significantly from 3:1 ($p > 0.5$). The mutation appears to be inherited as a single recessive nuclear gene. We designated the observed SGE0802 phenotype as *xavs* — *xantha-variegata-viridescens* seedlings.

Table 1. Segregation of F_2 hybrids from crosses of line SGE0802 (*xavs*) with parental line SGE.

Hybrid	F ₂ segregation phenotype		Segregation 3:1 χ^2	Probability P
	<i>Xavs</i>	<i>xavs</i>		
SGE0802 x SGE	184	64	0.086	0.7–0.8
SGE x SGE0802	152	56	0.410	0.5–0.6

We tried to localize *xavs* on the pea genetic map. Line SGE0802 was crossed with a number of tester lines, but we failed to find linkage of *xavs* with any of the genetic markers involved. *Xavs* is not positioned near genes *i*, *r*, *d*, *wb*, *k*, *st*, *b*, *a*, *lc*, *gp*, *wlo*, *oh*, *p*, *pl*. The question concerning its location on pea gene map remains unanswered.

In *Arabidopsis thaliana*, two genes, *var1* and *var2* produce the variegation pattern, very similar to the pattern observed in *xavs* pea plants, but the *Arabidopsis* phenotype is constitutive during all plant ontogenesis (7). *Arabidopsis* variegated mutations also form three sector types: white, pale green and dark green, again as was observed in the *xavs* mutant. In *Arabidopsis* mutants, dark green sectors contain normal chloroplasts whereas plastids in the white sectors are vacuolated with obscure inner membrane structures. Cells of pale green sectors contain plastids of both types. Proplastids in shoot apical meristems in *var* *Arabidopsis* mutants are normal, and abnormal plastids lacking inner membrane structures begin to form at later stages (3). *Var* genes belong to a small gene family of 12 nuclear genes coding for special chloroplast-localized FtsH metalloproteases, homologous to bacterial metalloproteases and responsible for degradation of photodamaged proteins within the photosynthetic machinery. Without this protease activity photosynthetic complexes become corrupted and that leads to irreversible chloroplast degradation (8).

As was shown by scanning electron microscopy, the pea shoot apical meristematic region involves well differentiated primordium for 4–5 nodes (9). Pea seeds possess a well-developed embryo, so we can suggest that it also contains well-expanded primordium for the 4–5 first leaves of the future seedling. In *Arabidopsis* FtsH genes are stage-specific and only two loci from 12 can produce variegated phenotypes similar to *xavs*. We can suggest, that *xavs* is a homolog of one of the FtsH-family representatives and is expressed specifically during seed development and maturation, at the stage of initiation of the 1st lateral meristem primordium formation. The mutant allele *xavs* produces a non-functional protease product, but this product can migrate into proplastids and bind with inner thylakoid membranes where normal metalloproteases are usually located. A little bit later, during formation of the 4th and 5th node meristems, expression of the other FtsH genes begin and producing two opposite FtsH gradients along primordium 1–5. At primordium 5 the concentration of functional FtsH metalloproteases becomes enough to result in normal plastid development. The concentration during initiation of the 2nd – 4th primordia is at a threshold level and that leads to a variegated phenotype appearance where some plastids may or may not develop normally. Certainly, pea gene *xavs* may produce some factor other than FtsH metalloprotease, but the general mechanism of variegated phenotype origin must be the same.

References

1. Von Wiren N., Mori S., Marschner H. and Romheld V. 1994. *Plant Physiology* 106:71–77.
2. Miura E., Kato Y. and Sakamoto W. 2010. *Journal of Experimental Botany* 61:2433–2445.
3. Sakamoto W., Tamura T., Hanba-Tomita Y., Sodmergen and Murata M. 2002. *Genes to Cells* 7:769–780.
4. Blixt S., 1972. *Agri Hortique Genetica* 30:1-293.
5. Lamprecht H. 1974. *Monographie der Gattung Pisum*. Steiermarkische Landesdruckerei, Graz, Austria.
6. Pisum Genetics Association Genelist: <http://www.jic.ac.uk/GERMPLAS/pisum/Zgc4g.htm>
7. Martinez-Zapater J.M. 1993. *J. Hered.* 84:138–140.
8. Sakamoto W. 2003. *Genes Genet. Syst.* 78:1-9.
9. Gourlay C.W., Hofer J.M.I. and Ellis T.H.N. 2000. *The Plant Cell* 12:1279–1294.

A new chlorophyll gene (*xach*, *xantha chlorescens*) in *Pisum sativum* L. located on LG II

Świąćicki, W.K.,
Gawłowska, M.
and Nawrot, Cz.

Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland

Induced mutations substantially increase the range of genetic variation in *Pisum* (eg. new genes *orp*, *art*, *nec*, *lum* etc.). Numerous accessions maintained in the Polish *Pisum* Gene Bank are mutations induced at the Plant Experiment Station at Wiatrowo (1, 2). Some of these, such as the one discussed in this paper, were obtained after treatment of both physical and chemical mutagens.

Dry seeds of cv. Paloma (Wt 3527) were treated with 200 rNf +0.014% NEU, and a chlorophyll mutant from the terminalis group was selected in the M₂ generation. During germination and initial growth (up to 4-5 leaves) mutant plants are gold-yellow-green (Figure 1A) and could be used as an ornamental pea. After this initial growth the plant becomes green and is fertile (Figure 1B). The phenotype is clearly different from other described chlorophyll mutations of this group. The mutant has been included in the Polish pea collection under the name *xantha-chlorescens* (accession number Wt 10889).



Figure 1. Mutant plants of cultivar Paloma showing chlorosis during early vegetative growth (A) and recovery of normal green foliage color (B).

This line was crossed to following testerlines with gene markers: Wt 11540 – A, *wb*, *Pgm-p* (LG II); *gp*, *tl*, *Acp1* (LG V); *Aat-m*, *Skdh*, *Est1*, *Est-2* (LG VII); Wt 11288 – A (LG II); *st*, *b*, (LGIII) and Wt 15860 – A (LG II); *creep*, *ce* (LG V). Segregation of the mutation in the F₂ in 1999 showed no deviations from an expected monohybrid segregation (Table 1). The gene symbol, *xach*, is suggested for the *xantha-chlorescens* mutation in the type line Wt 10889.

Table 1. Monohybrid segregation for the investigated gene *xach* (*xanta-chlorescens*) and gene markers in the linkage group II in F_2 populations of the linkage test crosses.

Cross no.	Parents	Gene	Dom	Rec	Total	χ^2
K. 2022	Wt 11540 x Wt 10889	<i>Xach</i>	70	25	95	0.09
		<i>A</i>	74	17	91	1.94
K. 2024	Wt 11288 x Wt 10889	<i>Xach</i>	72	22	94	0.13
		<i>A</i>	72	10	82	7.17
K. 2026	Wt 10889 x Wt 15860	<i>Xach</i>	69	25	94	0.13
		<i>A</i>	62	11	73	3.84
K. 2718	Wt 10886 x Wt 16054	<i>Xach</i>	97	29	126	0.26
		<i>Rms3</i>	79	20	99	1.22
K. 2795	Wt 10886 x Wt 15869	<i>Xach</i>	108	38	146	0.08
		<i>A</i>	88	41	129	3.16
		<i>Pal</i>	118	20	138	8.13
K. 2894	Wt 10886 x Wt 11300	<i>Xach</i>	89	30	119	0.00
		<i>A</i>	89	30	119	0.00
		<i>Crd</i>	95	24	119	1.48
K. 3012	Wt 3838 x Wt 10888	<i>Xach</i>	174	55	229	0.12
		<i>A</i>	161	60	221	0.54
		<i>Lf</i>	161	55	216	0.02

Analyses of the dihybrid segregation in the three mapping populations showed independent assortment between *xach* and all genes listed in Table 2. In contrast, substantial deviations were observed for *Xach-A* with the joint *chi* square from 21.4 to 50.8 and *Cr-O* values 8.27 in K.2022, 12.3 in K.2024 and 15.5 in K.2026. This suggests localization of *xach* on LG II (3).

Table 2. Distribution of phenotypes in F_2 populations (Wt 10889 *xach* type line x testerlines) and the linkage test for the new gene.

Testerline (Cross no.)	Pair of genes	DD	Dr	rD	rr	Total	Joint <i>chi</i> square	Cr-o (+S.E) (per cent)		Phase
Wt 11540 (K. 2022)	<i>Xach-Gp</i>	53	16	14	4	87	0.01	49.2	8.11	R
	<i>Xach-Tl</i>	52	18	17	2	89	1.98	35.1	9.16	R
	<i>Xach-Wb</i>	54	15	15	3	87	0.22	45.4	8.44	R
	<i>Xach-Ac</i>	57	9	16	5	84	0.08	46.7	8.48	R
	<i>Xach-Aat-m</i>	32	7	9	2	50	0.00	50.2	10.6	R
	<i>Xach-Skdh</i>	28	10	9	2	49	0.30	43.3	11.5	R
	<i>Xach-Acpl</i>	27	11	10	1	49	1.82	69.1	12.8	C
	<i>Xach-Pgm-c</i>	32	7	10	1	50	0.50	39.1	11.8	R
	<i>Xach-Pgm-p</i>	25	11	9	1	46	1.72	68.7	13.2	C
	<i>Xach-Est-1</i>	30	9	9	2	50	0.12	45.8	11.1	R
	<i>Xach-Est-2</i>	28	11	9	2	50	0.45	58.0	11.5	C
Wt 11288 (K.2024)	<i>Xach-St</i>	56	16	10	5	87	0.84	57.7	7.32	R
	<i>Xach-B</i>	51	17	2	2	72	1.22	64.7	7.29	R
Wt 15860 (K.2026)	<i>Xach-Creep</i>	51	17	11	6	85	0.73	56.8	7.50	R
	<i>Xach-Ce</i>	38	15	1	2	56	1.98	70.9	7.45	R

K.2024 and K.2026 single plants were selected from F_2 segregating populations and lines were multiplied with linked genes *xach-A* in repulsion phase (Wt 10888 and Wt 10886, respectively). These lines were crossed to additional LG II marker lines: Wt 16054 (*rms3*), Wt 15869 (*pal*), Wt 11300 (*crd*) and Wt 3838

(lf) (Table 1.). Analysis of the dihybrid segregation in the F_2 confirmed that *xach* is linked with *A*, although *Cr-O* values in repulsion were larger (from 19.6 to 29.3) than in coupling (Table 3). No linkages of *xach* with markers *rms3*, *pal* and *crd* were observed. However, in the population K.3012 substantial deviations from the expected dihybrid segregation were stated for three gene pairs: *Xach-A*, *Xach-Lf* and *A-Lf*. *Cr-O* values suggest the following gene order: *Xach-14-Lf-19-A*.

Table 3. Distribution of phenotypes in F_2 populations (*xach* lines x testerlines) and the linkage test for the new gene.

Cross no.	Pair of genes	DD	Dr	rD	rr	Total	Joint <i>chi</i> square	<i>Cr-O</i> (+S.E) (per cent)		Phase
K. 2022	<i>Xach-A</i>	68	2	5	14	89	50.8	8.27	3.07	C
K.2024	<i>Xach-A</i>	68	3	4	7	82	31.4	12.3	3.92	C
K.2026	<i>Xach-A</i>	59	5	3	6	73	21.4	15.5	4.68	C
K.2718	<i>Xach-Rms-3</i>	73	20	6	1	99	1.27	43.0	8.10	R
K.2795	<i>Xach-A</i>	68	40	20	1	129	9.26	19.6	8.40	R
	<i>Xach-Pal</i>	89	19	29	1	138	3.26	26.0	7.84	R
	<i>A-Pal</i>	74	13	35	6	128	00.0	50.3	6.65	C
K.2894	<i>Xach-A</i>	61	28	28	2	119	8.31	25.6	8.47	R
	<i>Xach-Crd</i>	69	20	26	4	119	1.11	41.1	7.52	R
	<i>A-Crd</i>	83	6	12	18	119	40.0	16.4	3.78	C
K.3012	<i>Xach-A</i>	113	55	48	5	221	10.3	29.3	6.07	R
	<i>Xach-Lf</i>	111	54	50	1	216	19.5	14.0	6.64	R
	<i>A-Lf</i>	138	16	22	38	214	65.2	18.9	3.03	C

References

1. Świącicki W.K. 1984. *Pisum* Newslet. 16: 84-86.
2. Świącicki W.K. 1998. Crop Gene Resources. Nation. Conf. Puławy: 53-54.
3. Weeden N.F., T.H.N. Ellis, G.M. Timmerman-Vaughan, W.K. Świącicki, S.M. Rozov and V.A. Berdnikov. 1998. *Pisum* Genetics 30: 1-4.

Use of Supernodulating Mutants in Pea Breeding

Sidorova, K.K.

Russian Academy of Sciences – Siberian Branch, Novosibirsk, Russia

Our genetic studies of symbiotic pea mutants have shown that the *Pisum* genome has multiple genes controlling the following traits: (1) capability of symbiosis with *Rhizobium* nodule bacteria; (2) the number and efficiency of root nodules; (3) nitrogen fixation rate intensity, assessed from nitrogenase activity; (4) duration of active nitrogen fixation; and (5) root biomass accumulation. We conclude that the macrosymbiont plays the key role in the genetic control of nitrogen fixation in nodules and that breeding of legume macrosymbionts for greater efficiency of their symbiosis with rhizobia is promising given that rhizobia are present in any substrate or soil, in the field or in a greenhouse.

Supernodulating mutants deserve special attention of breeders as a means for increasing nodulation and nitrogen fixation. However, these mutants suffer from low productivity. The first attempts to involve supernodulating mutants in breeding were done with soybean, despite being unsuccessful (1). To test the feasibility of utilizing supernodulating mutants in legume breeding as tools for intense nitrogen fixation, we performed large-scale field and greenhouse experiments with pea and investigated the expression of the *nod4* and *nod3* supernodulating genes against various genetic backgrounds (2). The results were used in the development of a breeding program for improving the efficiency of the legume–rhizobium symbiosis (Figure 1).

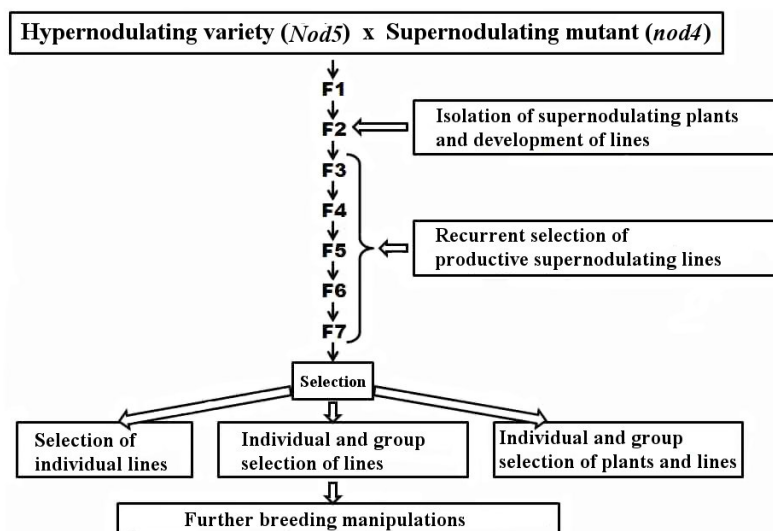


Figure 1. Breeding program for increasing nitrogen fixation in pea *Pisum sativum* L.



Figure 2. Pea roots: (1) supernodulating; (2) hypernodulating.

It is important to choose a proper variety, which should be highly productive and hypernodulating. Hypernodulation is characterized by large nodules, forming mainly in the upper and middle portions of roots. The nodules are considerably fewer in number, but larger than in supernodulating forms (Figure 2). Hypernodulation is governed by the dominant allele at the *Nod5* locus located on linkage group (LG) III (3, 4). The pea germplasm collection at Novosibirsk includes pea mutants and varieties with hypernodulation for *Nod5*. The supernodulation in mutant K301, used in our study, has the recessive allele at the *nod4* locus located in LG V (5).

Our method of breeding for elevated nitrogen fixation in pea was successfully applied to the development of the pea cultivar Druzhnaya.

Materials and methods

Supernodulating plants were selected in F_2 , and individual lines were raised. Productive lines with supernodulation and intense nitrogen fixation were recurrently selected in generations F_3 to F_7 . Performance and nodulation indices in mutant K301, cv. Druzhnaya, and recurrent lines are illustrated in Figure 3.

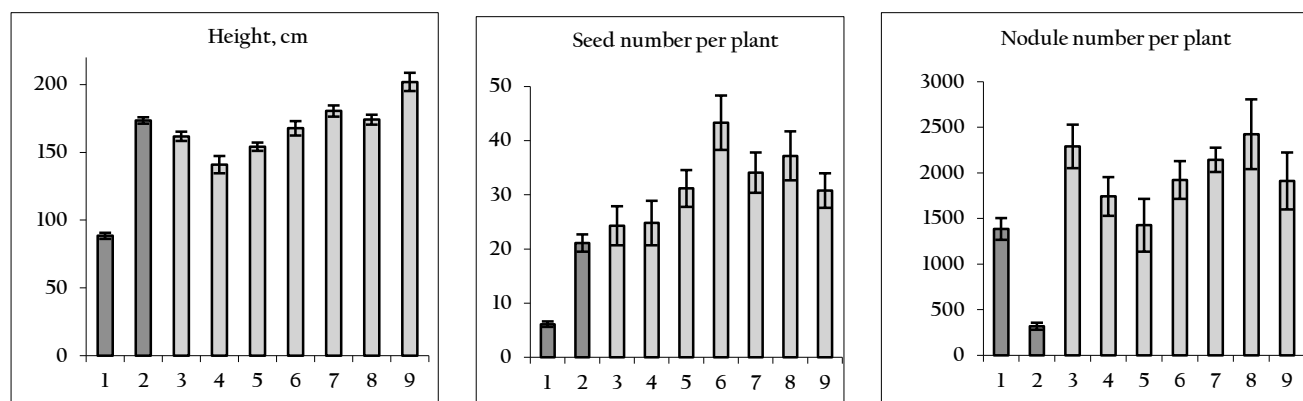


Figure 3. Recurrent F_7 lines obtained from crosses of cv. Druzhnaya to supernodulating mutant K301. Lane 1, K301; lane 2, Druzhnaya; and lanes 3–9, recurrent lines.

Results and discussion

The supernodulating mutant was both shorter and had less seed production than either Druzhnaya or the recurrent lines. The recurrent lines were slightly taller than Druzhnaya. Many recurrent lines had higher seed yield than Druzhnaya. The recurrent lines considerably outperformed Druzhnaya in symbiosis-related traits, i.e. number of nodules (Figures 3 and 4) and nitrogen fixation rate (Table 1).

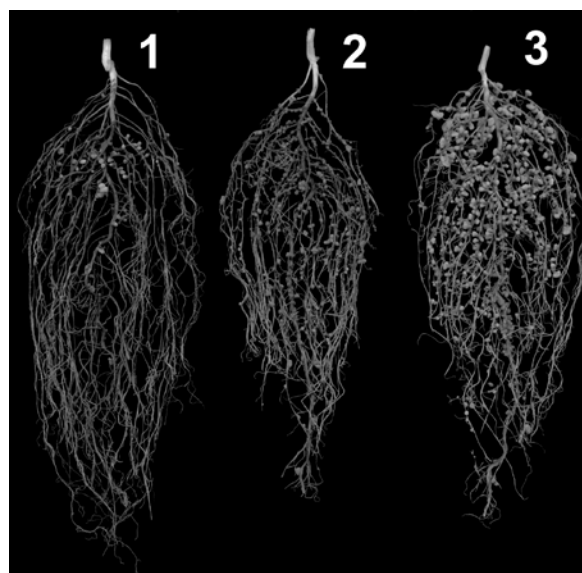


Figure 4. Representative examples of pea root biomass of cv. Druzhnaya (Nod5) (1), supernodulating mutant K301 (nod4) (2), and recurrent line K720 ultrasuper (nod4 Nod5) (3).

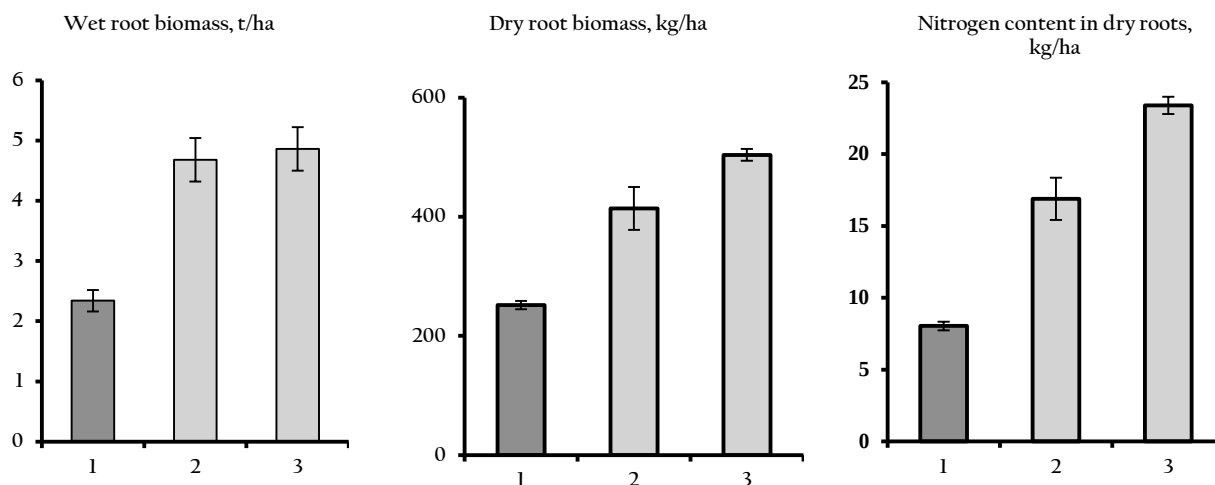
Table 1. Nitrogen fixation in pea cv. Druzhnaya and the recurrent lines.

Form	Nitrogenase activity, $\text{nmol C}_2\text{H}_4/\text{plant/h}$
cv. Druzhnaya	424
Supernodulating K301 mutant	1242
Recurrent lines:	
K301 × Druzhnaya	1123-1226
Druzhnaya × K301	2312-2380

Individual and group selection of recurrent lines was done in the F_7 . The lines were subjected to further breeding procedures. Similar results were obtained with cv. Novosibirskaya, also crossed to K301.

Conclusion

Legumes are good rotation crops. We have shown that broad expansion of the pea root system is possible based on results from the recurrent lines. All recurrent lines significantly surpassed cv. Druzhnaya in vegetative root biomass and nitrogen content (Figure 5).



The calculations were performed by the conventional method: 1,800,000 plants per hectare.

Figure 5. Accumulation of crude root biomass and nitrogen contents in pea forms: 1, cv. Druzhnaya; 2, 3, recurrent lines.

Our results indicate that forms approaching commercial varieties in performance and symbiosis parameters and even outperforming them in the accumulation of root biomass can be obtained by combining dominant and recessive alleles of different symbiosis-related genes. The cultivation of such forms will improve soil fertility and save mineral fertilizers, which is important with respect to environment protection and energy saving.

References

1. Bhatia C.R., Nichterlein K., and Maluszynski M. 2001 Euphytica, 120: 415-432.
2. Sidorova K.K., Shumny V.K., and Nazaryuk V.M. 2006 Symbiotic nitrogen fixation: genetic, breeding, ecological, and agrochemical aspects, Geo, Novosibirsk, P. 134 (in Russian).
3. Sidorova K.K. and Shumny V.K. 1997 Doklady Akademii Nauk 353(5):703-704. (in Russian).
4. Sidorova K.K., Shumny V.K., and Mishchenko, T.M. 1999 Doklady Akademii Nauk 367(6):851-852 (in Russian).
5. Sidorova K.K. and Uzhintseva L.P. 1994 Doklady Akademii Nauk, 1994 336(6):847-849 (in Russian).

In situ evaluation of a *Pisum sativum* subsp. *elatus* population from the valley of the river Pčinja in southeast Serbia

Ćupina, B.¹, Zlatković, B.²,
Smýkal, P.³, Mikić, A.⁴,
Jajić, I.¹,
Zeremski-Škorić, T.⁴
and Medović, A.⁵

¹University of Novi Sad, Field and Vegetable Crops, Novi Sad, Serbia

²University of Niš, Department of Biology and Ecology, Niš, Serbia

³Palacký University at Olomouc, Dept. of Botany, Olomouc, Czech Republic

⁴Institute of Field and Vegetable Crops, Novi Sad, Serbia

⁵Museum of Vojvodina, Novi Sad, Serbia

Introduction

There is a taxon within the genus *Pisum* L. colloquially called *elatus*, commonly considered a subspecies within common pea (*P. sativum* L.), namely *P. sativum* L. subsp. *elatus* (Steven ex M. Bieb.) Asch. & Graebn. (1). In further text referred to as *P. elatus*, it is regarded as a direct progenitor of cultivated pea (2), although with not satisfactorily described process of domestication yet. In a wider sense from an ecological viewpoint, *P. elatus* is a Mediterranean element with native distribution in Albania, Algeria, Azerbaijan, Bulgaria, Croatia, Cyprus, Egypt, France, FYR of Macedonia, Georgia, Greece, Iran, Israel, Italy, Lebanon, Libya, Montenegro, Morocco, Portugal, Russia, Romania, Serbia, Spain, Syria, Tunisia, Turkey and the Ukraine (3).

The first records on the presence of *P. elatus* in the flora of Serbia come from the second half of nineteenth century by the father of modern Serbian biology and ecology, Josif Pančić (4). This species is known to be distributed in several regions of the country and most abundantly in the south. Also, there are reports of a white-flowered variety (var. *albiflorum*) unlike the usual violet-flowered ones (5). However, existing information on the distribution of *P. elatus* in Serbia is incomplete. Recently, this pea taxon has been rediscovered in the southeastmost regions of Serbia, namely in the area of the upper Pčinja river and quite close to the modern border between Serbia and Former Yugoslav Republic of Macedonia (Figure 1). In that area, *P. elatus* inhabits the lower zone of northerly exposed slopes of Mount Kozjak, on the left side of the valley, amidst the slopes covered by termophilous submediterranean forests and scattered scrub vegetation on siliceous rocky soil (6).

The aim of this preliminary research was to carry out an initial *in situ* evaluation of the population of *P. elatus* growing in the upper Pčinja River.

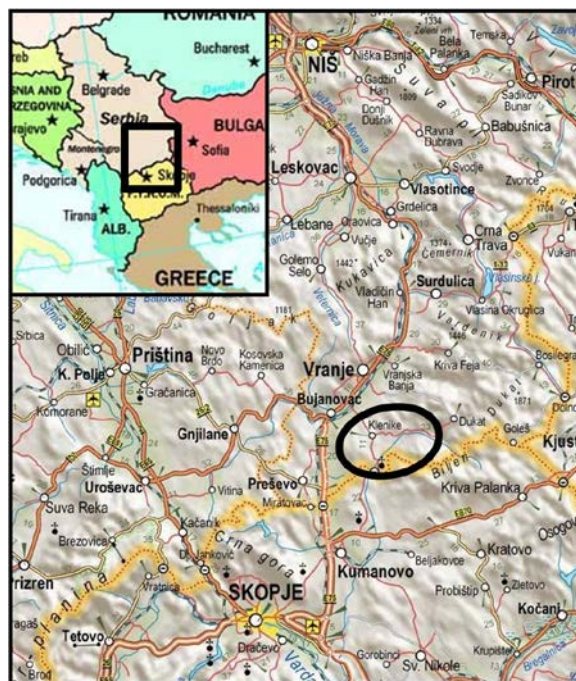


Figure 1. The upper flow of the river Pčinja, where *P. elatus* was re-discovered in 2009.

Materials and methods

Two expeditions were made in 2011 with an ultimate goal of collecting samples for a more detailed *in situ* evaluation of the *P. elatius* population from the region of the Pčinja River (Fig. 1), with emphasis on agronomic characteristics, such as yield components, and chemical composition, stressing the nutritive value.

The first expedition was performed on 28 May 2011, targeting the same area where *P. elatius* was re-discovered in 2009. A group of several plants was found on the edge between the foothills of the Mount Kozjak, between the forest and the meadow in the very vicinity of the Pčinja River and the monastery of Saint Prohor Pčinjski from 11th century. The plants were in the full bloom stage and forming the first pods (Figure 2), in further text referred to as Stage 1. Not far from this group, up the slopes of the Mount Kozjak and amidst the dense canopy of trees and shrubs, another population was found in the same stage, with plants either creeping over the rocky ground or climbing up and hanging from branches.

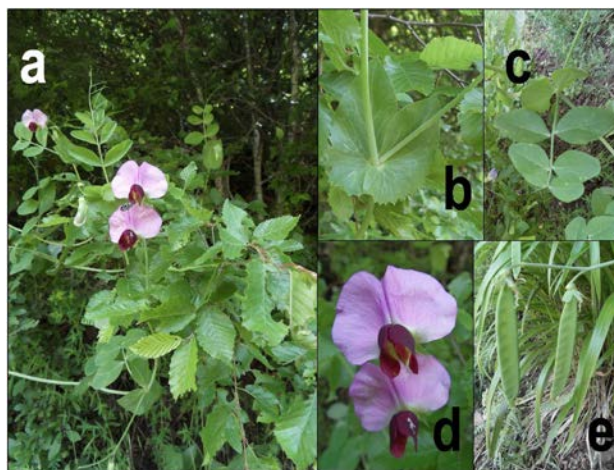


Figure 2. Some features of the *P. elatius* population from the upper flow of the river Pčinja in southeast Serbia in 2011: (a) a plant climbing up a beech tree; (b) stipules; (c) leaflets; (d) flowers; (e) pods.

By several plants growing on inaccessible slopes between these two groups, it may be assumed that the first group is a progeny of the second one, developing from the seeds that gradually rolled downwards, although this must remain speculative until a comparative research of these two populations is done with molecular markers. Fourteen plants were sampled in total from both groups and used for the analysis of agronomic characteristics and chemical composition related to forage.

The second expedition on 25 June 2011 visited the same population of *P. elatius* near the Pčinja River. Intact, both groups were in full maturity of the oldest pods, with a few pods already shattering their seeds. This was referred to as Stage 2. The pods stood erect and followed the direction of their peduncles, unlike the hanging ones of cultivated pea. Another set of fourteen plants was sampled from both groups and for the analysis of agronomic characteristics and chemical composition of the seeds. At least a dozen plants were left for regeneration of the population.

The agronomic analysis comprised determining the main yield components including (Stage 1) stem length (cm), number of stems (plant⁻¹), stem mass (g plant⁻¹), leaf mass (g plant⁻¹), plant mass (g plant⁻¹), stem proportion (%) and leaf proportion (%), as well as (Stage 2) stem length (cm), number of stems (plant⁻¹), number of pods (plant⁻¹), number of grains (plant⁻¹), stem mass (g plant⁻¹), leaf mass (g plant⁻¹), pod mass (g plant⁻¹), seed mass (g plant⁻¹), plant mass (g plant⁻¹) and harvest index.

The analysis of chemical composition at both stages comprised determining the content (g kg⁻¹) of crude protein, crude fat, crude fiber, neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, crude ash and nitrogen-free compounds (NFC).

The data related to the agronomic characteristics in both stages are presented as minimum, maximum and average values. The results of the chemical composition and nutritive value in both stages were processed using Statistica 8.0 software, with analysis of variance (ANOVA) performed and a Fisher's Least Significant Difference (LSD) test used at *P* = 0.05.

Results and discussion

The analysis of agronomic characteristics of the *P. elatius* population that are of interest in its cultivated relatives revealed wide variation of certain morphological traits that are also important forage yield characteristics (Table 1). It is neither possible nor appropriate to compare these results with those obtained in related species under field conditions, although *P. elatius* grew together with many other plants, mostly grasses and legumes, such as vetches (*Vicia* spp.). At any rate, the average dry plant mass in the stage of full bloom and forming the first pods may provide a basis that this pea taxon has good potential for forage production. In addition, the leaf proportion was higher than 50%, indicating the potential for good forage quality.

Table 1. *In situ* forage related characteristics of the *P. elatius* population from the upper Pčinja River in southeast Serbia in 2011 (Stage 1).

	Stem length (cm)	Number of stems (plant ⁻¹)	Stem mass (g plant ⁻¹)	Leaf mass (g plant ⁻¹)	Plant mass (g plant ⁻¹)	Stem proportion	Leaf proportion
Minimum	31	1	4.48	7.70	12.18	0.13	0.48
Maximum	192	8	35.14	63.42	98.56	0.52	0.87
Average	68	3.8	11.04	22.44	33.48	0.33	0.67

Average stem length and stem number did not change significantly from Stage 1 to Stage 2 (Table 2). Average seed mass (5.60 g plant⁻¹) may be regarded as rather high, being perhaps another indicator of the domestication level of this pea taxon. Its harvest index, the ratio between the mass of mature seeds and the remainder of the mature and dry plant parts is also considerably high for a wild relative of a crop plant (0.38).

Table 2. *In situ* seed related characteristics of the *P. elatius* population from the upper Pčinja River in southeast Serbia in 2011 (Stage 2).

Stage 2	Stem length (cm)	Number of stems (plant ⁻¹)	Number of pods (plant ⁻¹)	Number of grains (plant ⁻¹)	Stem mass (g plant ⁻¹)	Leaf mass (g plant ⁻¹)	Pod mass (g plant ⁻¹)	Grain mass (g plant ⁻¹)	Plant mass (g plant ⁻¹)	Harvest index
Minimum	52	1	2	12	2.82	1.28	0.61	2.46	7.17	0.34
Maximum	168	7	11	74	14.34	5.25	2.35	15.71	37.65	0.42
Average	72	4.1	4.3	27.3	5.52	2.38	1.24	5.60	14.74	0.38

The average protein content (Table 3) in *P. elatius* forage dry matter (201 g kg⁻¹) is slightly higher than in typical forage pea cultivars (7) and is closer to those in vetches (8). The average crude protein content in grain dry matter (343 g kg⁻¹) is much higher than in dry pea cultivars or other important grain legume crops (9, 10) and close to another interesting legume crop wild relative (11), *Vavilovia formosa* (Stev.) Fed. As expected, crude protein content in Stage 1 is highest in leaf tissue. When combined with the already mentioned high leaf proportion in total plant mass, it may be interesting for forage pea breeders. On the other hand, the content of crude fiber, together with its fractions like ADF, NDF and lignin, was higher than in typical perennial forage legume crops (12).

Table 3. *In situ* nutritive value of the *P. elatius* population dry matter (g kg⁻¹) from the upper Pčinja river in southeast Serbia in 2011.

	Crude protein	Crude fat	Crude fiber	NDF	ADF	Lignin	Crude ash	NFC
Stage 1								
Stem	115	8	483	619	532	136	61	333
Leaf	244	25	265	353	320	62	76	391
Plant	201	19	337	441	390	86	71	372
LSD _{0.05}	32	6	123	45	35	15	6	29
Stage 2								
Stem	69	12	528	674	587	128	88	303
Leaf	146	41	291	324	307	76	131	390
Pod	98	5	437	573	415	93	70	391
Grain	343	-	188	242	205	19	-	-
LSD _{0.05}	67	11	211	69	55	27	33	31

Conclusions

This preliminary research will be continued on several levels. This newly found population of *P. elatius* will be maintained *ex situ* and further evaluated within the field collection of the Institute of Field and Vegetable Crops in Novi Sad. Also, this population will be monitored *in situ* and visited before the winter, to check if there is any fall growth of new plants, as well as in early spring next year, with the same purpose. In addition, more locations will be searched for *P. elatius*, thus verifying previous reports of its presence in the flora of Serbia.

Acknowledgements

This research is a part of the projects I68 SEELEGUMES within the European Union FP7-supported SEE-ERA.NET Plus programme, *Towards Comprehensive Pea Germplasm Management for Future Use* within the AEGIS programme of the Bioversity International and TR31016 and TR31024 of the Ministry of Education and Science of the Republic of Serbia.

The authors are grateful to Mike Ambrose and T. H. Noel Ellis for their advice and encouragement and Svetlana Antanasović and Đorđe Krstić for their assistance.

References

1. Davis, P. H. (1970) In: Flora of Turkey and East Aegean Islands 3, University of Edinburgh, Edinburgh, UK, pp. 370-373.
2. Zeven, A.C. and Zhukovsky, P.M. (1975) Dictionary of Cultivated Plants and Their Centres of Diversity. Centre for Agricultural Publishing and Documentation, Wageningen.
3. Maxted, N. and Ambrose, M. (2001) In: Plant Genetic Resources of Legumes in the Mediterranean. Kluwer, Dordrecht, the Netherlands, pp. 181-190.
4. Pančić, J. (1874) Flora Kneževine Srbije. Državna štamparija, Belgrade.
5. Kojić, M. (1972) In: Flora SR Srbije 4. Srpska Akademija Nauka i Umetnosti, Belgrade, Serbia, pp. 386-388.
6. Zlatković, B., Mikić, A. and Smýkal, P. 2010. Pisum Genetics 42: 15-17.
7. Mihailović, V., Mikić, A., Erić, P., Vasiljević, S., Čupina, B. and Katić S. 2005. Biotechnology in Animal Husbandry 21:5-6:1: 281-285.
8. Mikić, A., Čupina, B., Katić, S. and Karagić, Đ. 2006. Ratarstvo i povrtarstvo / Field and Vegetable Crops Research 42: I: 91-103.
9. Mihailović, V., Mikić, A., Vasić, M., Čupina, B., Đurić, B., Duc, G., Stoddard, F.L., Hauptvogel, P. 2010. Ratarstvo i povrtarstvo / Field and Vegetable Crops Research 47: 27-32.

10. Mikić, A., Mihailović, V., Čupina, B., Đurić, B., Krstić, Đ., Vasić, M., Vasiljević, S., Karagić, Đ. and Đorđević, V. 2011. Food and Chemical Toxicology 49: 650-654.
11. Zeremski-Škorić, T., Mikić, A., Sarukhanyan, N., Vanyan, A., Akopian, J., Gabrielyan, I., Smýkal, P., Kenicer, G., Vishnyakova, M. and Ambrose, M. 2010. Pisum Genetics 42: 28-30.
12. Mihailović, V., Mikić, A., Čupina, B., Milić, D., Krstić, Đ., Katić, S., Vasiljević, S. and Karagić, Đ. 2011. Ratarstvo i povrtarstvo / Field and Vegetable Crops Research 48: 417-424.

Some agronomic aspects of the intercrops of semi-leafless and normal-leafed dry pea cultivars

Antanasović, S.¹, Mikić, A.²,
Ćupina, B.¹, Krstić, Đ.¹,
Miailović, V.², Erić, P.²
and Milošević, B.¹

¹University of Novi Sad, Faculty of Sciences, Novi Sad, Serbia

²Institute of Field and Vegetable Crops, Novi Sad, Serbia

Introduction

Pea (*Pisum sativum* L.) is present in both wild and agricultural floras of Serbia and other Balkan countries. The subspecies *arvense* is traditionally cultivated as a forage crop, while the subspecies *sativum* is a widespread garden plant. During the last two decades of the last century, the first dry pea cultivars, mostly of Canadian and Czechoslovakian origin, were introduced in the then Yugoslav, and now Serbian, agriculture. Today, dry pea, usually known among the Serbian farmers as *protein* pea, is grown mostly for feed, with an average harvested area of between 20,000 ha and 25,000 ha (1). The only institution involved in dry pea breeding in Serbia today is the Institute of Field and Vegetable Crops in Novi Sad (2), with nearly 20 cultivars registered in the country and a moderately large pea collection (3).

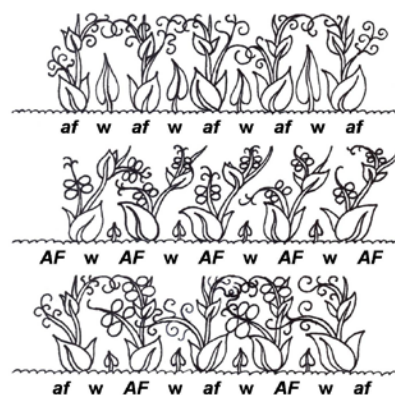
Dry pea shows good agronomic performance when used as a companion crop in the establishment of a perennial forage legume such as red clover (4). Especially suitable are 'semi-leafless' cultivars, with all leaflets of the compound pea leaf transformed into tendrils. There are many advantages in comparison with normal-leafed dry pea cultivars. Among these is better penetration of sunlight into a pea stand and all green parts of a plant are included in photosynthesis. Additionally, there is enhanced airflow and which results in less favorable conditions for developing pea diseases and pests (5). Recently, an idea was developed to attempt intercropping semi-leafless (*af*) and normal-leafed (*AF*) pea cultivars for both forage and dry grain production, with a possible reduction of weeds (*W*) in the former and lodging in the latter (Figure 1), resulting in better utilization of both stand area and volume.

The initial objective of this research is to assess the agronomic performance of the intercrops of semi-leafless and normal-leafed dry pea cultivars as affected by stand density and their proportion in the mixture.

Materials and methods

Small-plot trials were carried out during 2010 and 2011 at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi, in the vicinity of Novi Sad on a slightly carbonated chernozem soil. The trial included three stand densities, namely 80 plants m⁻², 100 plants m⁻² and 120 plants m⁻². Within each stand density, there were five proportions of one semi-leafless (*af*) : one normal-leafed (*AF*) cultivars: 100% : 0%; 75% : 25%; 50% : 50%; 25% : 75% and 0% : 100%. The semi-leafless cultivar 'Partner' and the normal-leafed cultivar 'Dukat' were both developed at the Institute of Field and Vegetable Crops and registered in Serbia in 2007. Both cultivars are characterized by a short vine length, on average from 45 cm to 60 cm in both. The two cultivars mature at approximately the same time and have very uniform maturity. The size of a trial plot was 5 m², with three replicates. The weeds were controlled by the application of 1 l ha⁻¹ an appropriate post-sowing and pre-emergence herbicide, Prometrin®. The plots

Figure 1. Theoretical aspects of intercropping semi-leafless and normal-leafed pea cultivars.



were not inoculated with rhizobia, since chernozem is rich in its strain specialized for pea. Inorganic fertilizer containing 45 kg ha⁻¹ of phosphorus (P) and potassium (K) each was applied in the preceding fall.

In both trial years, all intercrops were sown in mid-March, as soon as the field conditions allowed, and harvested during the second half of June when the seeds in the first pods had reached full maturity. Since the determinate stem growth in both cultivars and a prominent maturing infirmity, there were no green pods at this stage. Each plot was harvested by hand. Each cultivar was separated from the other and they were combined separately with a Wintersteiger Nurserymaster. The grain yield of all intercrops was measured at a seed moisture content of 14%.

The economic reliability of grain yield in each intercrop was determined by calculating the Land Equivalent Ratio (LER) as (6):

$$LER = af_{IC} / af_{SC} + AF_{IC} / AF_{SC},$$

where af_{IC} is the grain yield of the semi-leafless cultivar in the intercrop, af_{SC} is the grain yield of the semi-leafless cultivar in its sole crop, AF_{IC} is the grain yield of the normal-leafed cultivar in the intercrop and AF_{SC} is the grain yield of the normal-leafed cultivar in its sole crop.

The analysis of variance (ANOVA) of the results was performed using Statistica 8.0 software. Means were separated using Fisher's Least Significant Difference (LSD), $P = 0.05$, for a combined analysis of the two sole crops and the intercrop.

Results and discussion

There were significant differences in grain yields, both between different stand densities and different proportions of individual dry pea cultivars (Table 1). In general, both cultivars reacted positively to

Table 1. Average values of grain yield (kg ha⁻¹) and Land Equivalent Ratio in the intercrops of semi-leafless and normal-leafed dry pea cultivars for 2010 and 2011 at Rimski Sanki.

Stand density (plants m ⁻²)	Treatment	GY _{af}	GY _{AF}	GY _{IC}	LER
80	100% af + 0% AF	3438	0	3438	1.00
	75% af + 25% AF	2700	1050	3750	1.17
	50% af + 50% AF	2100	1300	3400	1.09
	25% af + 75% AF	1525	1675	3200	1.06
	0% af + 100% AF	0	2700	2700	1.00
100	100% af + 0% AF	4625	0	4625	1.00
	75% af + 25% AF	3350	1225	4575	1.27
	50% af + 50% AF	2325	1575	3900	1.20
	25% af + 75% AF	1425	1788	3213	1.10
	0% af + 100% AF	0	2250	2250	1.00
120	100% af + 0% AF	4975	0	4975	1.00
	75% af + 25% AF	3475	1275	4750	1.14
	50% af + 50% AF	2525	1513	4038	1.04
	25% af + 75% AF	1925	2113	4038	1.12
	0% af + 100% AF	0	2863	2863	1.00
Average	100% af + 0% AF	4346	0	4346	1.00
	75% af + 25% AF	3175	1183	4358	1.18
	50% af + 50% AF	2317	1463	3779	1.09
	25% af + 75% AF	1625	1858	3483	1.09
	0% af + 100% AF	0	2604	2604	1.00
LSD _{0.05}		778			0.07

increasing their stand densities in both intercrops and their sole crops, from 80 plant m⁻² to 120 plant m⁻².

The two-year average grain yield in the sole crops of the semi-leafless cultivar ranged from 3438 kg ha⁻¹ at 80 plants m⁻² to 4975 kg ha⁻¹ at 120 plants m⁻². The two-year average grain yield in the sole crops of the normal-leafed cultivar ranged from 2250 kg ha⁻¹ at 80 plants m⁻² to 2863 kg ha⁻¹ at 120 plants m⁻². In a two-year average, the semi-leafless cultivar had significantly higher yield than the normal-leafed cultivar. This agrees with previous research in the same conditions (7) and may be basically explained as the consequence of a better balance between grain yield components in comparison to those in the normal-leafed cultivar.

In all intercrops, the semi-leafless cultivar had significantly higher proportion in the total dry grain yield than the normal-leafed one. The averages ranged from 3175 kg ha⁻¹ to 1183 kg ha⁻¹ at 75% : 25% and from 2317 kg ha⁻¹ to 1463 kg ha⁻¹ at 50% : 50%. The normal-leafed cultivar had a higher proportion in the total dry grain yield when planted as 75% of the intercrop (1858 kg ha⁻¹), but not significant in comparison to the remaining 25% of the semi-leafless cultivar (1625 kg ha⁻¹).

The LER values were higher than 1 in all intercrops, regardless of the stand density and the proportion of individual components with different leaf types. However, the highest LER values, as affected by crop density, were in the intercrops of 75% of the semi-leafless and 25% of the normal-leafed cultivars, with 1.17, 1.27 and 1.14, respectively (Figure 2). This proportion proved economically most reliable in average as well, with its LER value of 1.18 significantly higher than 1.09 in both other proportions. It is noteworthy that the intercrops of semi-leafless and normal-leafed pea cultivars are reliable in forage production, as documented by another pioneer study in the same conditions (8).

Figure 2. An intercrop of the semi-leafless and normal-leafed dry pea cultivars at a ratio of 75% : 25% at Rimski Sani in 2011.



These results pose opportunities for further research. These two cultivars were intentionally chosen for intercropping due to similar growing habit and developmental stages. However, future research could provide a more stringent evaluation of intercropping by developing F₅- or F₆-derived near isogenic semi-leafless and normal-leafed varieties (9).

Conclusions

This initial study needs to be repeated over years and in diverse environments. However, this study documents the intercropping of semi-leafless and normal-leafed dry pea cultivars as an alternative solution for enhanced grain production in temperate regions such as Serbia. Future research will target more detailed analysis of grain yield components in the intercrops and diverse physiological parameters, as well as numerous underground aspects such as plant-microbial interactions and nutrient dynamics.

Acknowledgements

This research is a part of the project TR-31016 of the Ministry of Education and Science of the Republic of Serbia.

References

1. Mihailović, V., Mikić, A., Čupina, B. and Erić, P. 2005. Grain Legumes 44: 25-26.
2. Mihailović, V., Mikić, A., Katić, S. and Karagić, Đ. 2009. Pisum Genetics 41: 26-28.
3. Mihailović, V., Mikić, A., Čupina, B., Krstić, Đ. and Milošević, B. 2009. Pisum Genetics 41: 44-45.
4. Čupina, B., Krstić, Đ., Mikić, A., Erić, P., Vučković, S. and Pejić, B. 2010. Turkish Journal of Agriculture and Forestry 34: 275-283.
5. Mikić, A., Mihailović, V., Čupina, B., Kosev, V., Warkentin, T., McPhee, K., Ambrose, M., Hofer, J. and Ellis, N. 2011. Ratarstvo i povrtarstvo / Field and Vegetable Crops Research 48: 275-284.
6. Kadžiulienė, Ž., Šarūnaitė, L. and Deveikytė, I. 2011. Ratarstvo i povrtarstvo / Field and Vegetable Crops Research 48: 183-188.
7. Mihailović, V., Mikić, A., Katić, S., Karagić, Đ., Milošević, B., Pataki, I. and Radojević, V. 2009. Plant Breeding and Seed Production 23: 51-56.
8. Čupina, B., Krstić, Đ., Antanasović, S., Erić, P., Pejić, B., Mikić, A. and Mihailović, V. 2010. Pisum Genetics 42: 11-14.
9. Mikić, A., Čupina, B., Mihailović, V., Krstić, Đ., Antanasović, S., Đorđević, V. and Perić, V. 2011. Book of Abstracts, EUCARPIA 29th Fodder Crops and Amenity Grasses Section Meeting.

Forage yields in the intercrops of pea with other cool season annual legumes

Krstić, Đ.¹, Mikić, A.²,

Ćupina, B.¹, Antanasović, S.¹,

Mihailović, V.², Erić, P.¹ and Pejić, B.¹

¹University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia

²Institute of Field and Vegetable Crops, Novi Sad, Serbia

Introduction

Pea (*Pisum sativum* L.) is an economically important plant species worldwide, especially in temperate regions. It is used in both human diets and animal feeding in the form of green forage, forage dry matter, forage meal, silage, haylage, immature pods, immature grains, mature grains and straw (1) and also plays a significant agronomic role through green manure and mulch. Pea is traditionally intercropped with cereals in many regions of Europe and the world, but also as a companion crop in establishing a perennial forage crop such as red clover (*Trifolium pratense* L.), alfalfa (*Medicago sativa* L.) or sainfoin (*Onobrychis viciifolia* Scop.), where it acts as a bioherbicide (2).

Today, pea is one of the most important annual forage legumes in Serbia and other Balkan and South East European countries. Recently, attempts have been made in Serbia to re-introduce neglected and underutilized annual legume crops such as faba bean (*Vicia faba* L.) (3), grass pea (*Lathyrus sativus* L.) (4), lentil (*Lens culinaris* Medik.) and bitter vetch (*Vicia ervilia* (L.) Willd.), as well as to introduce novel annual legume crops such as white lupin (*Lupinus albus* L.) (5), that could serve as supplements in providing animal husbandry with quality plant protein.

The aim of this study was to assess the possibility of intercropping pea with other temperate annual legumes for forage production.

Materials and methods

A small-plot trial was carried out during two pea growing seasons, from the fall 2009 to the spring 2011 on a chernozem soil at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi near Novi Sad. Treatments comprised several intercrop combinations of pea with other cool season annual legumes, as well as the intercrop of pea with contrasting leaf morphology. All treatments were designed according to the four basic principles of the mutual annual legume intercropping (6): 1) same time of sowing; 2) similar growth habit; 3) similar cutting time; and 4) combinations of crops with good standing ability (supporting crop) with others that are susceptible to lodging (supported crop). Seven treatments involving pea were included in the trials: 1) fall-sown forage pea + faba bean, 2) fall-sown semi-leafless dry pea + normal-leaved dry pea, 3) fall-sown semi-leafless dry pea + bitter vetch, 4) spring-sown forage pea + faba bean, 5) spring-sown forage pea + white lupin, 6) spring-sown semi-leafless dry pea + normal-leaved dry pea and 7) spring-sown semi-leafless dry pea + lentil (Figure 1). Each component of the treatments was also included in the trial as a pure stand.



Figure 1. Intercrops involving pea and other cool season annual legumes 2010 and 2011 at Rimski Šančevi: (fall-sowing) forage pea + faba bean (first row, left), semi-leafless dry pea + normal-leaved dry pea (first row, right), semi-leafless dry pea + bitter vetch (second row), (spring-sown) forage pea + faba bean (third row, left), forage pea + white lupin (third row, right), semi-leafless dry pea + normal-leaved dry pea (fourth row, left) and semi-leafless dry pea + lentil (fourth row, right).

All fall intercrops and sole crops were sown on 8 October 2009 and 15 October 2010, while all spring intercrops were sown on 2 March 2010 and 6 March 2011. Plot size was 5 m² and the experimental design was a split-plot with three replicates. The seeding rates in sole crops were 75 viable seeds m⁻² for fall- and spring-sown faba bean and white lupin, 120 viable seeds m⁻² for fall- and spring-sown forage and dry pea and 180 viable seeds m⁻² for bitter vetch and lentil. In all the intercrops, the seeding rates of each component in its sole crop were reduced by 50%. No rhizobia or inorganic fertilizers were added and no weed control was done. All the plots with sole crops were cut at the full bloom or early pod stage, while the intercrops were cut when the first intercrop component reached the full bloom or early pod stage.

The green forage yield in all intercrops was measured immediately after cutting. The forage dry matter yield in each was determined on the basis of forage dry matter proportion in the green forage samples taken after the cutting and dried until constant mass at a room temperature. The reliability of green forage yield in each intercrop was determined by calculating its Land Equivalent Ratio (LER_{GFY}) as (6):

$$LER_{GFY} = GFY(sg)_{IC} / GFY(sg)_{SC} + GFY(sd)_{IC} / GFY(sd)_{SC},$$

where GFY(sg)_{IC} is the green forage yield of the supporting component in the intercrop, GFY(sg)_{SC} is the green forage yield of the supporting component in its sole crop, GFY(sd)_{IC} is the green forage yield of the supported component in the intercrop and GFY(sd)_{SC} is the green forage yield of the supported component in its sole crop. In an analogous way, the LER for forage dry matter yield (LER_{FDMY}) was calculated.

The results were analyzed using Statistica 8.0 software. Analysis of variance (ANOVA) was performed and means were separated using Fisher's Least Significant Difference (LSD) test at $P = 0.05$.

Results and discussion

The seedling emergence in all the treatments was regular and provided the projected stand density. Average green forage yields in the pure stands of fall-sown forage pea (46.1 t ha⁻¹) and spring-sown faba bean (45.6 t ha⁻¹) were significantly higher compared to the other treatments, especially the spring-sown normal leaf dry pea (27.9 t ha⁻¹) and lentil (23.3 t ha⁻¹) (Table 1). Among the fall-sown treatments, the

Table 1. Average values of green forage yield (t ha⁻¹) and its Land Equivalent Ratio (LER_{GFY}) in the intercrops of pea with other cool season annual legumes for 2010 and 2011 at Rimski Šančevi.

Season	Treatment	Green forage yield			LER _{GFY}
		Supporting crop	Supported crop	Total	
Fall	Faba bean	38.3	-	38.3	1.00
Fall	Forage pea	-	46.1	46.1	1.00
Fall	Faba bean + forage pea	16.5	32.0	48.5	1.12
Fall	Semi-leafless dry pea	33.5	-	33.5	1.00
Fall	Normal-leafed dry pea	-	29.6	29.6	1.00
Fall	Bitter vetch	-	35.6	35.6	1.00
Fall	Semi-leafless dry pea + normal-leafed dry pea	22.1	17.9	40.0	1.26
Fall	Semi-leafless dry pea + bitter vetch	24.1	22.5	46.6	1.35
Spring	Faba bean	45.6	-	45.6	1.00
Spring	White lupin	41.2	-	41.2	1.00
Spring	Forage pea	-	39.2	39.2	1.00
Spring	Faba bean + forage pea	19.2	23.4	42.6	1.02
Spring	White lupin + forage pea	17.3	26.9	44.2	1.11
Spring	Semi-leafless dry pea dry pea	30.1	-	30.1	1.00
Spring	Normal-leafed dry pea	-	27.9	27.9	1.00
Spring	Lentil	-	23.3	23.3	1.00
Spring	Semi-leafless dry pea + normal-leafed dry pea	17.4	15.6	33.0	1.14
Spring	Semi-leafless dry pea + lentil	19.5	14.8	34.3	1.28
LSD _{0.05}		4.1			0.10

average two-year green forage yield ranged from 40.0 t ha⁻¹ in the intercrop of semi-leafless and normal-leaved peas to 48.5 t ha⁻¹ in the intercrop of faba bean and forage pea. In the spring-sown treatments, the highest green forage yield was in the intercrop of white lupin and forage pea (44.2 t ha⁻¹), while the lowest green forage yield was in the intercrop of semi-leafless and normal-leaved peas (33.0 t ha⁻¹).

The intercrops of semi-leafless pea, both fall-sown and spring-sown, had significantly higher two-year average values than all other intercrops, especially 1.35 in the intercrop with bitter vetch and 1.28 in the intercrop with lentil. In comparison to some other cool season annual legumes that also may play the role of supported crop, such as grass pea, spring-sown forage pea had higher forage yields in the intercrops with faba bean and white lupin, as well as much lower values of LER_{GFY} when intercropped with both (7).

Overall, the two-year average forage dry matter yield (Table 2) followed the same trend as the green forage matter yield. Among the sole crop treatments, the average green forage yields in spring-sown faba bean (13.2 t ha⁻¹) were significantly higher compared to the other treatments, especially lentil (5.6 t ha⁻¹). In the fall-sown intercrops, the average two-year green forage yield ranged from 9.8 t ha⁻¹ in the intercrop of semi-leafless and normal-leaved peas to 12.5 t ha⁻¹ in the intercrop of faba bean and forage pea. Among the spring-sown intercrops, the intercrops of forage pea with faba bean and white lupin produced the highest green forage yield (both 11.0 t ha⁻¹), while the intercrop of semi-leafless and normal-leaved peas produced the lowest green forage yield (7.7 t ha⁻¹).

Table 2. Average values of forage dry matter yield (t ha⁻¹) and its Land Equivalent Ratio (LER_{FDMY}) in the intercrops of pea with other cool season annual legumes for 2010 and 2011 at Rimski Šančevi.

Season	Treatment	Forage dry matter yield			LER _{FDMY}
		Supporting crop	Supported crop	Total	
Fall	Faba bean	11.1	-	11.1	1.00
Fall	Forage pea	-	11.3	11.3	1.00
Fall	Faba bean + forage pea	4.6	7.8	12.5	1.11
Fall	Semi-leafless dry pea	8.5	-	8.5	1.00
Fall	Normal-leaved dry pea	-	7.3	7.3	1.00
Fall	Bitter vetch	-	8.9	8.9	1.00
Fall	Semi-leafless dry pea + normal-leaved dry pea	5.4	4.4	9.8	1.24
Fall	Semi-leafless dry pea + bitter vetch	5.9	5.6	11.5	1.32
Spring	Faba bean	13.2	-	13.2	1.00
Spring	White lupin	11.5	-	11.5	1.00
Spring	Forage pea	-	9.4	9.4	1.00
Spring	Faba bean + forage pea	5.4	5.6	11.0	1.00
Spring	White lupin + forage pea	4.5	6.5	11.0	1.08
Spring	Semi-leafless dry pea dry pea	7.2	-	7.2	1.00
Spring	Normal-leaved dry pea	-	6.6	6.6	1.00
Spring	Lentil	-	5.6	5.6	1.00
Spring	Semi-leafless dry pea + normal-leaved dry pea	4.0	3.7	7.7	1.11
Spring	Semi-leafless dry pea + lentil	4.5	3.6	8.0	1.26
LSD _{0.05}		0.8			0.11

The intercrops of semi-leafless pea, both fall-sown and spring-sown, had significantly greater two-year average values compared to the other intercrops, namely 1.32 in the intercrop with bitter vetch and 1.26 in the intercrop with lentil. In comparison to the results of other trials with mutual annual legume intercrops, such as those including warm-season legumes such as soybean (*Glycine max* (L.) Merr.) and few *Vigna* species, the intercrops of pea with other cool season annual legumes had higher values of LER_{FDMY} (8).

Conclusions

Depending on individual cases, intercropping various types of pea, such as forage pea and dry pea with afila and normal leaves, with other cool season annual legumes may lead to higher forage yields and an economical justification by high LER values and better utilization of natural resources. In comparison to the traditional intercropping pea and other annual legumes with cereals for forage production, the mutual intercropping of annual forage legumes provides farmers with high quality forage richer in protein. Further research on the same subject will focus on forage quality aspects, such as the crude protein and crude fiber content in forage dry matter and other less examined issues such as forage yield components and crop physiology.

Acknowledgements

This research is a part of the project TR-31016 of the Ministry of Education and Science of the Republic of Serbia.

References

1. Mihailović, V., Mikić, A., Erić, P., Vasiljević, S., Čupina, B. and Katić S. 2005. Biotechnology in Animal Husbandry 21:5-6:1: 281-285.
2. Čupina, B., Mikić, A. and Krstić, Đ. 2009. Grain Legumes 52: 9.
3. Mihailović, V., Mikić, A., Vasić, M., Čupina, B., Đurić, B., Duc, G., Stoddard, F.L. and Hauptvogel, P. 2010. Ratarstvo i povrtarstvo / Field and Vegetable Crops Research 47: 27-32.
4. Mikić, A., Mihailović, V., Čupina, B., Đurić, B., Krstić, Đ., Vasić, M., Vasiljević, S., Karagić, Đ. and Đorđević, V. 2011. Food and Chemical Toxicology 49: 650-654.
5. Mikić, A., Mihailović, V., Čupina, B., Đorđević, V. and Stoddard, F.L. 2010. Ratarstvo i povrtarstvo / Field and Vegetable Crops Research 47: 21-26.
6. Čupina, B., Mikić, A., Stoddard, F.L., Krstić, Đ., Justes, E., Bedoussac, L., Fustec, J. and Pejić, B. 2011. In: Lichtfouse, E. (ed), Sustainable Agriculture Reviews 7: Genetics, Biofuels and Local Farming Systems. Springer, Dordrecht, 347-365.
7. Čupina, B., Mikić, A., Mihailović, V., Krstić, Đ. and Đurić, B. 2009. Grain Legumes 54: 32.
8. Mikić, A., Đorđević, V., Perić, V., Čupina, B., Mihailović, V., Srebrić, M. and Krstić, Đ. 2010. Proceedings, XII International Symposium on Forage Crops of Republic of Serbia, Kruševac, Serbia, 26-28 May 2010, 2, 269-275.

Intercropping pea with eight cereals for forage production

Mihailović, V.¹, Mikić, A.¹,

Kobiljski, B.¹, Čupina, B.²,

Antanasović, S.², Krstić, Đ.² and Katanski, S.²

¹Institute of Field and Vegetable Crops, Novi Sad, Serbia

²University of Novi Sad, Novi Sad, Serbia

Introduction

Pea (*Pisum sativum* L.) is widely distributed in both wild and agricultural flora of Serbia and other Southeast European countries. *P. sativum* subsp. *sativum* var. *arvense* (L.) Poir., appears as a weed in cereals, especially fall-sown wheat in southeastern Serbia (1). It is also cultivated for forage production and has been successfully used for developing fall-sown cultivars of forage pea highly resistant to low temperatures (2).

Cultivated area of forage pea in Serbia has been about 4000 ha for several decades (3). Forage pea is traditionally used in fall-sown mixtures with cereals (4) such as common wheat (*Triticum aestivum* L. subsp. *aestivum*), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.) and triticale (*Triticosecale* spp.). The seed mixture of forage pea and cereals depends on local recommendations and is 50:50 in Lithuania (5) and France (6) and 75:25 (pea:cereal) in Serbia (7) and Bulgaria (8).

The goal of this research was to assess the potential of pea intercrops with various cereals for forage production in temperate regions of Serbia.

Materials and methods

A small-plot trial was carried out at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi during the growing seasons of 2009/2010 and 2010/2011 (Table 1) and on a chernozem

Table 1. Average monthly temperatures (°C) and monthly rainfall (mm) during the 2009/2010 and 2010/2011 growing seasons.

Temperature	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Average
2009/2010	12	9	3	0	2	7	13	17	20	9
2010/2011	10	10	1	0	0	6	13	17	18	8
Long-term	12	6	2	-1	2	6	11	17	20	8
Rainfall	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Sum
2009/2010	83	64	96	73	65	38	71	95	174	605
2010/2011	67	44	66	29	35	28	23	65	61	375
Long-term	43	50	48	37	32	38	47	59	85	374

soil (Table 2). It included intercrops of forage pea with eight cereals, namely einkorn (*Triticum monococcum* L.), emmer (*Triticum turgidum* L. subsp. *dicoccon* (Schränk) Thell.), spelt (*Triticum aestivum* L. subsp. *spelta* (L.) Thell.), durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.), common wheat, barley, oat and triticale. The sole crops of each intercrop species were also included in the trial.

Table 2. Chemical composition of the chernozem soil at Rimski Šančevi in 2009.

pH (H ₂ O)	pH (KCl)	N (%)	P ₂ O ₅ (mg 100 g ⁻¹)	K ₂ O (mg 100 g ⁻¹)	CaCO ₃ (%)	Humus (%)
7.9	7.41	0.196	17.99	21	5.61	2.97

In both trial years, all sole crops and their intercrops were sown in the second half of October, with plot size of 5 m² and three replicates. All sole crop treatments were harvested at full bloom or first pod formation in pea and in the full flag leaf stage for the cereal crops. The intercrop treatments were harvested when the first crop of the mixture reached the desired stage (9). In the majority of treatments both component crops reached the desired stage concurrently.

The green forage yield in all intercrops and sole crops was measured directly after cutting. Forage dry matter yield was determined after allowing the harvested samples to dry to a constant mass in a drier at 105 °C. The agronomic and economic reliability of green forage yield and forage dry matter production in each intercrop was determined by calculating their Land Equivalent Ratio (LER_{GFY} and LER_{FDMY}) according to (10):

$$LER_{GFY} = GFY(p)_{IC} / GFY(p)_{SC} + GFY(c)_{IC} / GFY(c)_{SC},$$

where $GFY(p)_{IC}$ is the green forage yield of pea in the intercrop, $GFY(p)_{SC}$ is the green forage yield of pea in its sole crop, $GFY(c)_{IC}$ is the green forage yield of a cereal in the intercrop and $GFY(c)_{SC}$ is the green forage yield of a cereal in its sole crop. Similarly, LER_{FDMY} was calculated.

The results were analyzed using Statistica 8.0 software, with analysis of variance (ANOVA) performed and a Fisher's Least Significant Difference (LSD) calculated at $P = 0.05$.

Results and discussion

There were significant differences in the two-year average green forage yield among both sole crops of pea and cereals and their intercrops (Table 3).

Table 3. Average green forage yield ($t\ ha^{-1}$) in sole crop (GFY_{SC}) and intercrop (GFY_{IC}) treatments of pea (GFY_P) and cereals (GFY_C) in 2009/2010 and 2010/2011 at Rimski Šančevi.

Sole crop	GFY_{SC}	Intercropping	GFY_P	GFY_C	GFY_{IC}	LER_{GFY}
Pea	43.5	-	-	-	-	-
Einkorn	34.5	Pea + einkorn	19.5	18.0	37.5	0.97
Emmer	51.0	Pea + emmer	9.0	37.5	46.5	0.94
Spelt	52.5	Pea + spelt	7.5	43.5	51.0	1.00
Durum wheat	34.5	Pea + durum wheat	28.5	12.0	40.5	1.00
Common wheat	42.0	Pea + common wheat	13.5	34.5	48.0	1.13
Barley	55.5	Pea + barley	7.5	48.0	55.5	1.04
Oat	36.0	Pea + oat	13.5	34.5	48.0	1.27
Triticale	45.0	Pea + triticale	16.5	30.0	46.5	1.05
C.V.						
$LSD_{0.05}$	4.8	$LSD_{0.05}$	4.8	0.09		

The average green forage yield in the intercrops ranged from $37.5\ t\ ha^{-1}$ in pea + einkorn to $55.5\ t\ ha^{-1}$ in pea + barley, confirming that in temperate conditions barley produces the highest forage yields, although with poorer quality (11). The largest proportion of pea was in its intercrop with durum wheat ($28.5\ t\ ha^{-1}$), followed by einkorn ($19.5\ t\ ha^{-1}$). The two-year average green forage yield in pea sole crop treatments was comparable to previous data under the same conditions (12). The intercrops of pea with einkorn, emmer, spelt and durum were not economically justified with LER_{GFY} values either lower or equal to 1.0. The intercrop of pea with oat had a significantly higher LER_{GFY} value (1.27) than the other seven intercrops.

In general, the two-year average forage dry matter yield (Table 4) followed similar trends as the two-year average forage dry matter yield. In sole crops, barley ($11.2\ t\ ha^{-1}$), spelt ($11.1\ t\ ha^{-1}$), emmer ($11.0\ t\ ha^{-1}$) and pea ($10.7\ t\ ha^{-1}$) had significantly higher forage dry matter yield in comparison to the remaining four cereals. The two-year average forage dry matter yield in the intercrops varied between $8.5\ t\ ha^{-1}$ in pea + einkorn and $11.5\ t\ ha^{-1}$ in pea + barley, the latter being lower than at the same pea and barley ratio in the temperate regions of North America (13). The forage dry matter proportion of each crop may differ slightly in a pure stand compared to an intercrop. For this reason, the values of LER_{FDMY} were slightly different than LER_{GFY} , with a maximum in the pea + oat intercrop (1.23) and a minimum in the pea + spelt intercrop (0.97).

Table 4. Average forage dry matter yield ($t\ ha^{-1}$) in sole crop ($FDMY_{SC}$) and intercrop ($FDMY_{IC}$) treatments of pea ($FDMY_P$) and cereals ($FDMY_C$) in 2009/2010 and 2010/2011 at Rinski Šančevi.

Sole crop	$FDMY_{SC}$	Intercropping	$FDMY_P$	$FDMY_C$	$FDMY_{IC}$	LER_{FDMY}
Pea	10.7	-	-	-	-	-
Einkorn	7.1	Pea + einkorn	4.6	3.9	8.5	0.98
Emmer	11.0	Pea + emmer	2.1	8.8	10.9	1.00
Spelt	11.1	Pea + spelt	1.8	8.9	10.7	0.97
Durum wheat	7.0	Pea + durum wheat	6.7	2.6	9.3	1.00
Common wheat	9.5	Pea + common wheat	3.2	7.7	10.8	1.11
Barley	11.2	Pea + barley	1.8	9.7	11.5	1.03
Oat	7.7	Pea + oat	3.2	7.2	10.4	1.23
Triticale	9.2	Pea + triticale	3.9	6.3	10.2	1.05
C.V.						
$LSD_{0.05}$	0.9	$LSD_{0.05}$	0.9	0.10		

Conclusions

This study confirmed that the traditional practice of intercropping pea with common wheat, barley, oat and triticale have the greatest potential for forage production in comparison to less traditional or forgotten crops such as durum wheat, spelt, einkorn and emmer. However, this study should be continued with a more detailed study on forage dry matter quality, with emphasis on crude protein and crude fiber as well as with all important underground aspects of intercropping, primarily plant-microbial interactions and nutrient availability.

Acknowledgements

This research is a part of the project TR-31016 of the Ministry of Education and Science of the Republic of Serbia and the EU project SEELEGUMES I68 within the SEE-ERA.NET plus program and under the auspices of the EU Seventh Framework Programme (FP7).

References

1. Mikić, A. and Čupina, B. 2011. pers. comm.
2. Mihailović, V. and Mikić, A. 2010. Proceedings, XII International Symposium on Forage Crops of Republic of Serbia, Kruševac, Serbia, 26-28 May 2010, 1, 81-90.
3. Mihailović, V., Mikić, A. and Čupina, B. 2007. Biotechnology in Animal Husbandry 23:5-6:1: 573-581.
4. Mikić, A., Čupina, B., Katić, S. and Karagić, Đ. 2006. Ratarstvo i povrtarstvo / Field and Vegetable Crops Research 42:I: 91-103.
5. Kadžiulienė, Ž., Šarūnaitė, L. and Deveikytė I. 2011. Ratarstvo i povrtarstvo / Field and Vegetable Crops Research 48: 183-188.
6. Bedoussac, L. and Justes, E. 2010. Plant and Soil 330: 37-54.
7. Mihailović, V., Čupina, B., Erić, P. and Trifunović, T. 1993. Ratarstvo i povrtarstvo / Field and Vegetable Crops Research 21: 517-523.
8. Angelova, S. and Sabeva M. 2011. pers. comm.
9. Mihailović, V., Mikić, A., Katić, S. and Karagić, Đ. 2009. Pisum Genetics 41: 26-28.
10. Čupina, B., Krstić, Đ., Antanasović, S., Erić, P., Pejić, B., Mikić, A. and Mihailović, V. 2010. Pisum Genetics 42: 11-14.
11. Mihailović, V., Erić, P. and Mikić, A. 2004. Grassland Science in Europe 9: 457-459
12. Mikić, A., Mihailović, V., Čupina, B., Đorđević, V., Milić, D., Duc, G., Stoddard, F.L., Lejeune-Hénaut, I., Margot, P. and Hanocq E. 2011. Euphytica 180: 57-67.
13. Strydhorst, S.M., King, J.R., Lopetinsky, K.J. and Harker, K.N. 2008. Agronomy Journal 100:182-190.

Can we reconstruct the most ancient words for pea (*Pisum sativum*)?

Mikić, A.

Institute of Field and Vegetable Crops, Novi Sad, Serbia

Introduction

Pea (*Pisum sativum* L.) primarily originated in the Near East and subsequently diversified in the Mediterranean and Ethiopia (1). The ancestors of modern humans came in touch with pea and other grain legumes and cereals in the earliest stages of their colonization of Eurasia, taking place between 69,000 and 59,000 years ago via Sinai (2). The fossilized starch grains in calculus of the skeletons from Shanidar Cave in Iraq show that Neanderthals cooked both cereals and grain legumes such as pea, chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* Medik.) about 46,000 years ago (3). The diets of European *Homo sapiens* hunter-gatherers during the last Ice Age were dominated mostly by lentil and bitter vetch (*Vicia ervilia* (L.) Willd.), as witnessed by the remains from Franchthi cave in Greece, from 11,000 BC (4), and the eastern coast of Spain, nearly 12,000 years ago (5). Pea is also one of the first cultivated crops in the Old World (6), with archaeological findings from present Syria dating back more than 10,000 years (7). The domestication of pea, lentil, bitter vetch, chickpea and faba bean (*Vicia faba* L.) could even predate cereals with firm evidence still missing (8).

A rather complex relationship between human genetics, ethnology and linguistics has already been attested (9). It was also demonstrated that a combined lexicological and etymological analysis may explain the paths of crop domestication, together with genomic and archaeobotanical approaches (10, 11). The first farmers in the Fertile Crescent descended from those who left Africa in the first wave of migration. It is still extremely uncertain how their ancestors diversified during their long voyage from Africa to Eurasia, as well as if all their descendents managed to survive the last Ice Age. There are theories that it is possible that at least one of these groups was given this unique opportunity. In that way, what is designed as Borean could be the hypothetical language of this group of humans surviving the Last Glacial Maximum from 18,000 to 15,000 years BC, welcoming the Upper Paleolithic between 17,000 and 15,000 years BC (12, 13) and subsequently developing into both Eurasian Palaeolithic hunter-gatherers and Near East Neolithic proto-farmers.

Since the speakers of the hypothetical Borean language are thought to be the progenitors of those who domesticated the first plants and animals, this essay aims to assess the possibility of reconstructing the Borean word or words denoting pea or other closely related grain legume crops.

Materials and methods

This palaeolinguistic venture by one plant scientist was carried out in several stages. The first step was to search all available printed and electronic etymological resources for the words denoting pea and its closest agronomic and botanical relatives, namely lentil, bitter vetch, chickpea or faba bean, in both recognized and controversial language families. This step was essentially assisted by the electronic etymological databases of the *Evolution of Human Language* project (14).

In the second stage, all the collected roots related to pea and other ancient Eurasian grain legumes were linked together on the scale of two suspected language megafamilies stemming directly from Borean (12, 15, 16). The first Borean-descending megafamily is Nostratic, comprising Indo-European, Uralic, Altaic, Kartvelian, Dravidian, Paleosiberian and Afroasiatic families (17, 18) and with a single ultimate ancestor called Proto-Nostratic (19). Another one is Dené-Daic (15, 20), consisting of Dené-Caucasian and Austric superfamilies (Figure 1).

The third and the final stage of this study was to examine the possibility of the existence of the Borean roots (13) related to either pea or other ancient Eurasian grain legumes.

The families comprising languages of humans who never left Africa, namely Khoisan, Niger-Kordofanian and Nilo-Saharan, and who left it in another migration, such as Indo-Pacific, Australian and Amerind, were not the subject of this research.



Figure 1. Widely recognized language families of the world (74): Eurasiatic comprises Indo-European, Uralic, Altaic and Paleosiberian.

Results

Indo-European. The diversity of the words denoting pea in modern Indo-European languages reveals several Proto-Indo-European roots related to pea and leguminous plants in general (21). Those in the majority of the modern Romance and Celtic languages, as well as in modern English, owe their origin primarily to the Latin *pisum*, denoting pea, and ultimately to the Proto-Indo-European **pis-*, meaning *to thresh* (22, 23). The words denoting pea in most of the modern Germanic languages originated from the Proto-Germanic **arwait*, denoting the same (23). Together with the Latin *ervum*, denoting bitter vetch, this Proto-Germanic root was a development of the Proto-Indo-European **erag^w(h)-*, denoting a kernel of leguminous plant (34, 49). All modern Slavic languages have the origin of their words denoting pea directly in the Proto-Slavic **gorxŭ*, denoting the same (24), and genuinely in the Proto-Indo-European **ghArs-*, denoting a leguminous plant (22, 23). The words denoting pea in the modern Baltic languages are derived from the Proto-Baltic **žirn-iā*, with the same meaning, and ultimately from the Proto-Indo-European **g^r(a)n-*, denoting grain (22, 23). The extinct Old Prussian and Old Indian reveal the Proto-Indo-European root **kek-*, denoting primarily pea (22, 23). This root began to denote chickpea in Latin and Armenian (24).

Other Nostratic families. There is no attested Proto-Uralic root directly associated with pea (25). However, the modern Uralic languages, spoken rather close to the supposed Uralic homeland in west Siberia (26), have their own and non-borrowed words denoting pea. They reveal the potential Proto-Uralic root, identical or similar to **kača*, denoting either a hole or cavity or a wooden vessel (25). The existing compendium of the words denoting pea in modern Altaic languages owes its origin to the Proto-Altaic **bŭkrV*, denoting pea, nut and cone (27). No Proto-Kartvelian root associated with pea has been identified yet (28). On the other hand, there is a Proto-Dravidian root **parup* denoting pea, pigeon pea (*Cajanus cajan* (L.) and other traditional grain legumes (29). There is also only one Proto-Afroasiatic root directly related to pea, **lay/w- ~, *ʔVll- ~, *w/yVlal-* (30).

Dené-Caucasian. There are two proto-roots denoting grain legumes in the Dené-Caucasian macrofamily (31). One of them is **hVwIV*, denoting faba bean and evolving into the Proto-Caucasian **hōwl[ā]*, where it denotes both bean and lentil (32), and the Proto-Basque **iθar̃*, denoting faba bean, pea or vetch (33). Another Proto-Dené-Caucasian root is **xqōr̃ʔā (~rĥ-)*, denoting a cereal and producing the Proto-Caucasian **qōr̃ʔā (~rĥ-)* where it denotes pea (32), the Proto-Sino-Tibetan **krā (-g-)*, denoting a kind of

grain (34) and the Common Burushaski **yarás*, denoting pea (35). No root directly associated to pea or other grain legumes was found in Proto-Yeniseian (36).

Austic. There are no attested Proto-Austic roots related to pea, but there are two roots denoting grain legumes, **ɖaj* and **tVk* (37).

Discussion

Nostratic. The Proto-Nostratic language is considered to have split into Proto-Indo-European, Proto-Uralic and Proto-Altaic by 5000 BC (19, 38). This may give a solid basis to search for the common roots related to pea within these three groups (17, 18). The Indo-European family is by far the most studied and the most reconstructed of all within the supposed Nostratic macrofamily. The attested richness of the words denoting pea and other ancient Eurasian grain legumes witness a rather high level of the agricultural knowledge of the speakers of Proto-Indo-European (24) in their original homeland in the Pontic-Caspian steppe (39). This agrees well with the recent archaeological evidence that the Proto-Indo-European society was largely based upon agriculture (40), unlike some initial views as dominantly pastoral (41). The Proto-Indo-European **kek-* and the Proto-Uralic **kača* could be the descendants of the single Proto-Eurasiatic root, with the latter being a memory of the times before the Proto-Uralic speakers inhabited the eastern slopes of the Urals and gradually give up agriculture in favor of fishing and hunting. Still there are no attested corresponding words in other Nostratic families. However, there are certain roots morphologically similar with these two in Proto-Indo-European and Proto-Uralic. They are the Proto-Altaic **k[ā]p'a*, denoting the act of covering or a sack (27) and the Proto-Kartvelian **kwrīm-*, denoting a kind of millet, and **kaqa*, related to grain, fruit stone or walnut (28). There is also an attested Proto-Nostratic root **kVSV*, denoting nut or acorn (42). All these roots have an obviously descriptive character and once could refer to pea and other grain legumes as well.

Dené-Daic. The Proto-Dené-Caucasian root related to pea, **xqōrʔā* (~*rñ-*), has more numerous descendants related to pea as compared to another, **hVwIV*, related to pea only in Basque. It is noteworthy there are at least two additional Proto-Dené-Caucasian roots denoting cultivated crops and with a similar morphological development as two described above. These are **Gūlʔe* (~**xG-*), denoting a kind of cereal, and **xq(w)VrV*, denoting both a kind of weed and cereal (43).

Borean

According to one of the most developed models (16), Borean split primarily into Nostratic and Dené-Daic branches, with the former comprising Eurasiatic and Afroasiatic groups and the latter consisting of Dené-Caucasian and Austric groups (Figure 2).



Figure 2. Map of the hypothetical evolution of Borean (solid lines) into Nostratic (dashed lines) and Dené-Daic (dotted lines) languages (16).

The Dené-Daic group was the first to separate from Borean, soon afterward dividing into Dené-Caucasian and Austric branches. Many see Dené-Caucasian as a group of remains of the older Paleolithic inhabitants of Eurasia that in many cases, such as the speakers of Basque, Caucasian and Burushaski, retreated to isolated pockets difficult to access and, therefore, easy to defend, remaining surrounded by the Nostratic proto-farmers coming from the Near East (44). In comparison to Nostratic, Dené-Caucasian is supported by weaker and less clear evidence, indicating that the geographic spread of Dené-Daic did occur before that of Nostratic (45). Recent genetic research shows that the Basque people have the most ancestral phylogeny in Europe based on the rare mitochondrial subhaplogroup U8_a, placing their origin in the Upper Paleolithic and in West Asia (46). The second of two expansion periods from Central Europe occurred around 15,000–10,000 years ago (46). This could suggest that the starting point of the internal differentiation of the Dené-Daic macrofamily truly was in West-Central Asia.

On the other hand, the Borean-speakers remaining in the Near East could be direct precursors of the Nostratic-speakers, with the original homeland within the Fertile Crescent. Their evolution is represented by the Kebaran (18,000–10,500 BC) and the Zarzian cultures (12,400–8500 BC), with the broad spectrum revolution associated with microliths, the use of the bow and arrow and the domestication of the dog (47). At the end of the Last Ice Age, the speakers of Nostratic continued to develop their material culture and definitely invented agriculture and animal husbandry. Proto-Nostratic could be spoken in Epipaleolithic, between 15,000 and 12,000 BC, and started to differentiate by 8000 BC. It covered the entire Fertile Crescent and beyond to Egypt and along the Red Sea to the Horn of Africa, bringing forth Proto-Afroasiatic (19). It spread into the Caucasus, producing Proto-Kartvelian, the Iranian Plateau, evolving into Proto-Elamo-Dravidian, and into Central Asia, where Proto-Eurasiatic further subdivided by 5000 BCE into Proto-Indo-European, Proto-Uralic and Proto-Altaic (19).

These results show that the words related to pea or other related ancient Eurasian grain legume crops are more or less satisfactorily attested in a few direct or indirect descendants of Borean. If we apply the identification of easily recognizable similarities as the very first step in any kind of comparative linguistics (48), is it feasible to connect the Proto-Indo-European **kek-*, the Proto-Uralic **kača* and the Proto-Dené-Caucasian **xqōrʔā (~rhi-)*? Could they be related to each other or is it a pure coincidence or a simple borrowing from each other? It is true that the roots for many crops were developed from either verbs or adjectives they had been very closely associated with (49, 50). There are two examples of the reconstructed Borean roots that are morphologically similar to what one may expect the Borean root denoting pea looked like. These are **KVCV* (Figure 3) and **KVNV* (Figure 4), with a hypothetical evolution developed in most of their direct and indirect languages. Are these two roots related to the

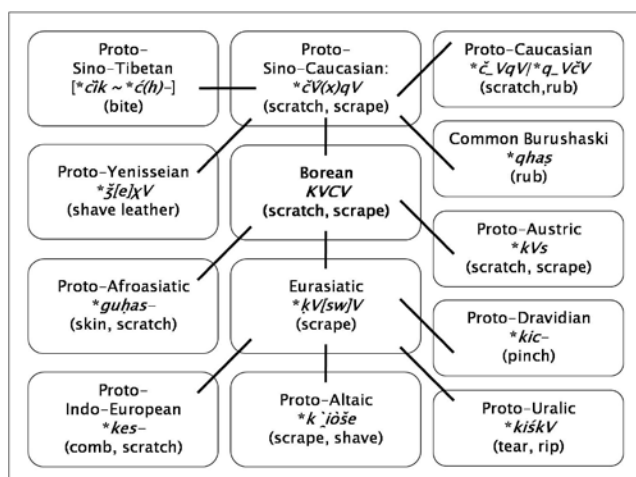


Figure 3. The evolution of the Borean root **KVCV* (32)

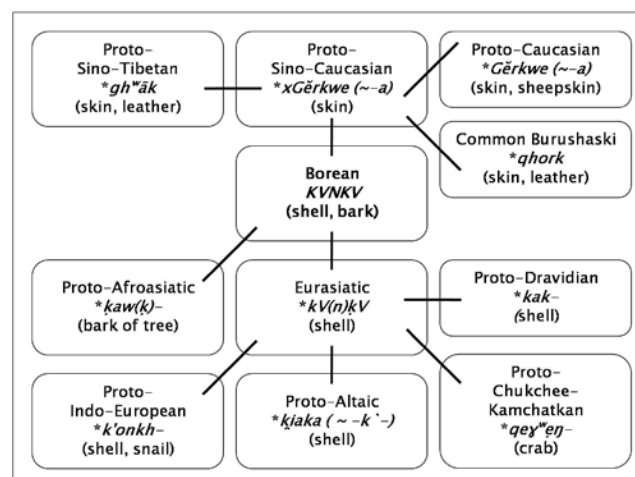


Figure 4. The evolution of the Borean root **KVNV* (32)

hypothetical and non-attested root related to pea in any way, regardless if the pea pods were simply gathered from wild flora or cultivated? Could it be that the verbs *to scratch* and *to scrape* were somehow associated with the very action of shelling pea seeds out of its pods? Also, could the nouns *shell* and *bark* refer to shell-like and skinny pea pods containing precious grains? This issue will surely remain open until a more detailed historical linguistic analysis is carried out. It is to be anticipated that its outcome could bring a root or the roots that were not necessarily associated with solely one species such as pea. Pea, lentil, chickpea and bitter vetch were surely easily recognizable for their shell-like and skinny pods with grains eaten cooked. In that way, this essay could equally assess the possibility of reconstructing the most ancient words not only for pea, but also for lentil, chickpea or bitter vetch: in the end, all of them could easily be considered very similar to each other or even identical in the everyday life of the ancient hunter-gatherers or proto-farmers of Eurasia.

Conclusions

Evidence of the use of pea in the diets of both Neanderthals and modern humans is confirmed with new findings. If the speakers of the Proto-Dené-Daic dominated Eurasia during the last stages of the Paleolithic, pea and other related ancient grain legumes were well known to them from wild flora, as they gathered them with cereals. On the other hand, speakers of the Proto-Nostratic, the first farmers of the Old World, domesticated pea together with other grain legumes and cereals. The existing linguistic thesaurus related to pea and other grain legumes in the descendants of the hypothetical Borean is rather rich and surely deserves more attention. A solid and deep reconstruction of the Borean words for pea and related grain legumes should be carried out, as it has been successfully done for other numerous everyday terms. Such linguistic attempts could assist in learning much more on the use of pea and other basic food crops by our ancestors and the individual steps in their domestication. This modest attempt represents an invitation to all those interested in crop history, namely archaeologists, archaeobotanists, plant scientists and linguists, to work together and develop multidisciplinary approaches offering answers to the issues related to the very dawn of mankind.

Acknowledgements

This research is a part of the projects TR-31024 of the Ministry of Education and Science and the SEELEGUMES within the EU programme SEE-ERA.NET Plus under the auspices of the EU Seventh Framework Programme (FP7).

To all the readers of *Pisum Genetics*, with deepest respect.

In memory of A.B. Popović, V.R. Đurić and S.A. Starostin.

References

1. Zeven, A.C. and Zhukovsky, P.M. 1975. Dictionary of Cultivated Plants and Their Centres of Diversity. Centre for Agricultural Publishing and Documentation, Wageningen, pp. 219.
2. Maca-Meyer, N., González, A.M., Larruga, J.M., Flores, C. and Cabrera, V.M. 2001. BMC Genomics 2: 13.
3. Henry, A.G., Brooks, A.S. and Piperno, D.R. 2011. Proceedings of the National Academy of Sciences of the United States of America 108: 486-491.
4. Farrand, W.R. 1999. Depositional History of Franchthi Cave. Indiana University Press, Bloomington, pp. 528.
5. Aura, J.E., Carrión, Y., Estrelles, E. and Pérez Jordà, G. 2005. Vegetation History and Archaeobotany 14: 542-550.
6. Zohary, D. and Hopf, M. 2000. Domestication of Plants in the Old World: The Origin and Spread of Cultivated Plants in West Asia, Europe and the Nile Valley. Oxford University Press, Oxford, pp. 316.

7. Tanno, K. and Willcox, G. 2006. *Vegetation History and Archaeobotany* 15: 197-204.
8. Kislev, M.E. and Bar-Yosef, O. 1988. *Current Anthropology* 29: 175-179.
9. Cavalli-Sforza, L.L. and Seielstad, M. (2001) *Genes, Peoples and Languages*. Penguin Press, London, pp. 227.
10. Diamond, J. and Bellwood, P. 2003. *Science* 300: 597-603.
11. Mikić, A., Medović, A., Čupina, B., Mihailović, V., Ignjatović-Čupina, A., Đorđević, V. and Kobiljski, B. 2011. *Nature Precedings* doi:10.1038/npre.2011.5837.1.
12. Gell-Mann, M., Peiros, I. and Starostin, G. 2009. *Journal of Language Relationship* 1: 13- 30.
13. Starostin, S. 2006. Long-range etymologies. The Tower of Babel. <http://starling.rinet.ru/cgi-bin/query.cgi?root=config&morpho=0&basename=\data\aura\globet>.
14. Evolution of Human Languages. 2005. An International Project on the Linguistic Prehistory of Humanity coordinated by the Santa Fe Institute. <http://ehl.santafe.edu/>.
15. Fleming, H.C. 1991. *Mother Tongue. Newsletter* V: 14.
16. Starostin, S. 2006. Borean Tree Diagram. The Tower of Babel. <http://starling.rinet.ru/images/globet.png>.
17. Greenberg, J. 2000. Indo-European and its Closest Relatives. The Eurasiatic Language Family. Grammar. Stanford University, Palo Alto, pp. 326.
18. Greenberg, J. 2002. Indo-European and its Closest Relatives. The Eurasiatic Language Family. Lexicon. Stanford University, Palo Alto, pp. 216.
19. Bomhard, A.R. 2008. *Reconstructing Proto-Nostratic: Comparative Phonology, Morphology, and Vocabulary*. Brill, Leiden, pp. 976.
20. Ruhlen, M. 2011. *Language Families of the World (after Greenberg)*. <http://www.meritttruhlen.com> meritt at meritttruhlen.com
21. Mikić, A. 2009. *Pisum Genetics* 41: 29-33.
22. Nikolayev, S. 2007. Indo-European etymology. The Tower of Babel. <http://starling.rinet.ru/cgi-bin/query.cgi?root=config&morpho=0&basename=\data\ie\piet>.
23. Pokorny, J. 1959. *Indogermanisches etymologisches Wörterbuch*. Francke, Bern.
24. Mikić, A. 2011. *Indogermanische Forschungen* 116: 60-71.
25. Starostin, S. 2005. Uralic etymology. The Tower of Babel. <http://starling.rinet.ru/cgi-bin/query.cgi?root=config&morpho=0&basename=\data\uralic\uralet>.
26. Janhunen, J. 2009. *Mémoires de la Société Finno-Ougrienne* 258: 57-78.
27. Starostin, S. 2006. Altaic etymology. The Tower of Babel. <http://starling.rinet.ru/cgi-bin/query.cgi?root=config&morpho=0&basename=\data\alt\altet>.
28. Starostin, S. 2005. Kartvelian etymology. The Tower of Babel. <http://starling.rinet.ru/cgi-bin/query.cgi?root=config&morpho=0&basename=\data\kart\kartet>.
29. Starostin, G. 2006. Dravidian etymology. The Tower of Babel. <http://starling.rinet.ru/cgi-bin/query.cgi?root=config&morpho=0&basename=\data\drav\dravet>.
30. Militarev, A. and Stolbova, O. 2007. Afroasiatic etymology. The Tower of Babel. <http://starling.rinet.ru/cgi-bin/query.cgi?root=config&morpho=0&basename=\data\semham\afaset>.
31. Mikić, A. 2011. *Mother Tongue* XVI (in press).
32. Starostin, S. 2005. North Caucasian etymology. The Tower of Babel. <http://starling.rinet.ru/cgi-bin/query.cgi?root=config&morpho=0&basename=\data\cauc\caucet>.
33. Bengtson, J. 2007. Basque etymology. The Tower of Babel. <http://starling.rinet.ru/cgi-bin/query.cgi?root=config&morpho=0&basename=\data\sinocauc\basqet>.
34. Starostin, S. 2005. Sino-Tibetan etymology. The Tower of Babel. <http://starling.rinet.ru/cgi-bin/query.cgi?root=config&morpho=0&basename=\data\sintib\stibet>.
35. Starostin, S. 2005. Burushaski etymology. The Tower of Babel. <http://starling.rinet.ru/cgi-bin/query.cgi?root=config&morpho=0&basename=\data\sinocauc\buruet>.
36. Starostin, S. 2005. Yeniseian etymology. The Tower of Babel. <http://starling.rinet.ru/cgi-bin/query.cgi?root=config&morpho=0&basename=\data\yenisey\yenet>.

37. Peiros, I. and Starostin, S. 2007. Austric etymology. The Tower of Babel. <http://starling.rinet.ru/cgi-bin/query.cgi?root=config&morpho=0&basename=\data\austr\austr>.
38. Renfrew, C. 1991. *Cambridge Archaeological Journal* 1: 3-23.
39. Anthony, D.W. 2007. *The Horse, the Wheel, and Language: How Bronze-Age Riders from the Eurasian Steppes Shaped the Modern World*. Princeton University Press, New Jersey, pp. 361.
40. Krell, K. 1998. In: *Archaeology and Language, II*, Routledge, London & New York, Chapter 11.
41. Gimbutas, M., Dexter, M.R. and Jones-Bley, K. 1997. *The Kurgan Culture and the Indo-Europeanization of Europe: selected articles from 1952 to 1993*. Institute for the Study of Man, Washington, pp. 404.
42. Starostin, S. 2005. Nostratic etymology. The Tower of Babel. <http://starling.rinet.ru/cgi-bin/query.cgi?root=config&morpho=0&basename=\data\nostr\nostret>.
43. Starostin, S. 2007. Sino-Caucasian etymology. The Tower of Babel. <http://starling.rinet.ru/cgi-bin/query.cgi?root=config&morpho=0&basename=\data\sinocauc\sccet>.
44. Ruhlen, M. 1998. Dene-Caucasian: A new linguistic family. In: *The Origins and Past of Modern Humans—Towards Reconciliation*, World Scientific, Singapore, pp. 231-246.
45. Ruhlen, M. 1994. *The Origin of Language: Tracing the Evolution of the Mother Tongue*. John Wiley & Sons, New York, pp. 239.
46. González, A.M., García, O., Larruga, J.M. and Cabrera, V.M. 2006. *BMC Genomics* 7: 124.
47. Weiss, E., Wetterstrom, W., Nadel, D. and Bar-Yosef, O. 2004. *Proceedings of the National Academy of Sciences of the United States of America* 101: 9551-9555.
48. Bengtson, J.D. and Ruhlen, M. 1994. In: *On the Origin of Languages: Studies in Linguistic Taxonomy*, Stanford University Press, Stanford, pp. 277-336.
49. Mikić-Vragolić, M., Mikić, A., Čupina, B., Mihailović, V., Vasiljević, S., Krstić, Đ. and Vasić, M. 2007. *Ratarstvo i povrtarstvo / Field and Vegetable Crops Research* 44: II: 91-96.
50. Mikić, A. and Stoddard, F.L. 2009. *Grain Legumes* 51: 34.

Registration of JI 3564 ‘Karl’s Longpod’ a Heritage Pea variety into the John Innes Pisum Collection

Mike Ambrose

John Innes Centre, Norwich, UK

In July 2010 correspondence and seeds of a heritage vegetable pea from a Mr. Karl Andrews of Lowestoft in Suffolk was received with a note to say that the line had been handed down to him by his Uncle Albert who was a keen lifelong amateur vegetable allotment grower (1920’s to the late 1980’s). Advice passed on by Uncle Albert was that “They are best grown from the 3rd week of March onwards” and “to allow plenty of air to circulate to avoid mildew”. Mr. Andrews still grows this line every year in his garden (Fig. 1a) and finds the peas to be “Very tender and sweet and produce a very good crop of big peas in most years”.

Figure 1. a. Karl Andrew standing next to plants of his ‘Longpod’ peas. b. mature pods.



The variety is a long vined main crop picking variety best suited for growing up a support. Seed of this line were grown out against wire and data recorded on a full range of morphological traits, the primary plant characteristics of which are summarised in Table 1. No direct or close match was found between this line and any other within the JI Pisum collection so its origin could not be established. Uncle Albert was reputed to “Never have bought seed and was much more likely to have obtained it from another

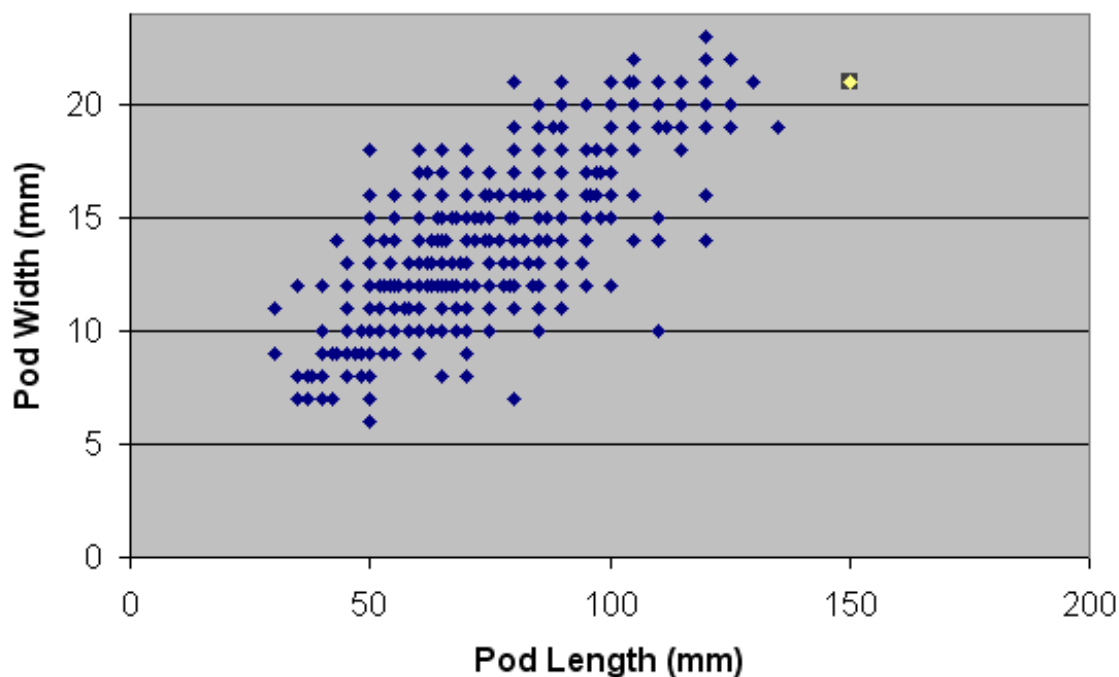
gardener.” The exact nature of this line cannot be established without comparing to a much broader range of comparable material which is not currently possible.

Table 1. Plant characteristics of JI 3564.

Internode length 150mm Large leaflets and stipules Nodes to flowering 14 Ht. to flowering 65cm Final plant height. 200cm White flower colour 1 flower per pedicel	Pod length 150mm (maximum) Pointed pod apex 12 ovules per pod Wrinkled and green cotyledons Seed weight 360mg Susceptible to powdery and downey mildew
---	---

The most notable characteristic of the line is the consistent production of very long pods (Fig1. b). The longest pod (150mm) sets a new maxima for pod length in the JI collection of any wrinkled seeded line. The next longest are those of Hurst Greenshaft (JI 430) at 120mm which received a Royal Horticultural Society Award of Gardening Merit (AGM) in 1993 and is still marketed by numerous seed companies today. In a plot of pod length versus pod width this line clearly stands out as an outlier within normal podded types (Fig. 2).

Figure 2. Pod width versus pod length for 886 accessions of normal pod types (none inflated or mangetout). JI 3564 is the data point highlighted as pale on the extreme right hand side.



On the basis of its outlier status for pod length the line has been registered into the JI Pisum collection as JI 3564 as a heritage variety and given the name ‘Karl’s Longpod’ and is available on request.

Abstracts and Short Papers from Oral Presentations at the North American Pulse Improvement Association (NAPIA) November 2-4, 2011 Meeting San Juan, Puerto Rico

Cool season food legume genome database: translating genomics for crop improvement

Bodah, E.T.^{1*}, Cheng, C.¹,
Main, D.¹, McGee, R.², Coyne, C.³,
Ficklin, S.¹, Jung, S.¹ and Zheng, P.¹

¹Washington State University, Pullman, WA USA

²USDA-ARS, GLGPRU, Pullman, WA USA

³USDA-ARS, PGIT, Pullman, WA, USA

*Presenter (eliane.bodah@email.wsu.edu)

Genomics-assisted breeding offers tools to optimize plant breeding efficiency by identify genes and/or markers related to traits of interest. The Cool Season Food Legume Genome Database (<http://www.coolseasonfoodlegume.org>) is being developed to assist in translating genomics into crop improvement. The main objective is to facilitate cool season food legume breeding and research by serving as a genomic, genetic and breeding data resource. Although databases exist for the model legumes, the Cool Season Food Legume Genome Database is specifically designed to collect and centralize data for pea, chickpea and lentil while using data from the model sequenced legumes for comparison and further curation. The database is built using Tripal which provides simplified site development by merging the power of Drupal, a popular web Content Management System (CMS), with that of Chado, a community derived database schema for storage of genomic, genetic and other biological data. Each page of the web site has a header bar composed of various categories for easy navigation: 'Home', 'About', 'Community', 'Crops', 'Maps', 'Tools', 'Search', 'Contact', 'Calendar', 'Publications', 'SCRI' and a link to join the 'Mailing List'. The 'Home', 'About', 'Community', 'Contact', 'Calendar', 'Publications' and 'SCRI' pages are self-explanatory and straight forward. The 'Crops', 'Maps', 'Tools' and 'Search' pages are described below. The 'Crops' page has a dropdown table of the currently available cool season food legume crops (pea, lentil and chickpea). For each crop, a unigene build for the publicly available ESTs along with the functional annotation data of the unigene set is available for users to browse. The functional annotation includes homologs in other model species, GO terms and KEGG pathway terms. The 'Crops' pages also have links to the genetic map data that can be viewed using the comparative map viewer, CMap, as well as links to NCBI and GRIN. The 'Tools' page provides SSR and BLAST servers, Medigaco GBrowse, Soybean GBrowse and Lotus GBrowse. The SSR server is an online tool that allows a user to upload sequences as a batch file, select the type of motifs they are interested in identifying and search the sequences for microsatellites. The results are returned by email for each SSR containing sequence. Sequences can be compared to the available datasets, such as the unigenes or the individual ESTs, using the BLAST server. The Genome Browsers are tools that allow the user to view the features of a genome such as predicted genes, markers and mapped transcripts. In the Cool Season Food Legume Genome Database, transcripts from pea, chickpea and lentil are mapped to regions/genes in the module legumes. This is useful for both function identification and fine mapping. The putative functions of transcribed genes can be assigned using various functional annotations. Functional annotation data of the EST unigenes can be browsed in web pages, downloaded in an excel file or queried using various categories in the EST/unigene 'Search' page. From the EST/unigene detail page, information on ESTs, gene ontology assignment, KEGG and InterProScan annotations along with homologs in other databases are available.

Sequences can also be downloaded in Fasta format. GO terms provide keywords of the type of processes/functions that the gene is involved in, KEGG annotation identifies potential pathways that the gene product belongs to, and the InterProScan analysis identifies functional domains in the gene product. These various annotations help build evidence for the function of a specific gene which can then be verified experimentally. Users can also query for sequences using a simple or advanced form. The advanced EST 'Search' page offers the option of EST or unigene search. Users can perform combinatorial search by name, assembly, sequence type, length or putative function. Registered users may acquire the collaborators status which allows them to view and analyze private research data. COS analysis, Gbrowse mapping, *M. trunculata* SSR analysis, and *P. sativum* SSR analysis are some of the private options stored under Research. Planned future development includes adding cool season food legume genome sequences as they become available, addition of more genetic maps and implementation of a publication search site, a breeder's survey form and an integrated breeder's toolbox such as the one we have developed for the genome database for Rosaceae (www.rosaceae.org).

KnowPulse: A breeder-focused web portal that integrates genetics and genomics of pulse crops with model genomes

Sanderson, L., Krilow, C., Vandenberg, A.,
Warkentin, T., Tar'an, B. and Bett, K.*

University of Saskatchewan, Saskatoon, SK, Canada

*Presenter

Current sequencing technologies have the ability to deliver vast amounts of genotypic data to unsuspecting plant breeders. The past few years has seen a huge increase in the amount of genotypic information now available for pulse crops, including pea, chickpea, lentil and common bean. These data are of limited use to breeders until associated with phenotypic information and recently several projects have been initiated to address this both at the CDC and abroad.

The trouble with vast amounts of data is how to store it and how to access it in a manner that is useful to breeders and geneticists. Traditionally, literature searches or word-of-mouth techniques have been used to find markers for use in breeding programs. However, as the number of available markers increases, this becomes less and less feasible. A clear need exists for resources, designed with the breeder's objectives and viewpoint in mind, that provide centralized access to marker details. The web portal KnowPulse (<http://pulse.usask.ca>) is being developed to address these needs. It is designed for pulse crop breeders and currently includes genotypic information and molecular markers for common bean, chickpea, lentil and field pea.

Within KnowPulse, each genomic marker has an information page meant to facilitate the integration of the marker into a breeding program. This includes details like the type and location of the marker and any known protocols for marker detection. A summary of observed alleles and the genotypes of all germplasm surveyed to date is also included on the marker page, allowing a researcher to determine the likelihood of a marker being polymorphic for a given variety or population.

Genotypic data can also be accessed through dynamic tables which allow researchers to select germplasm entries of interest. Filters are available allowing researchers to select markers that are polymorphic in specific germplasm, or specific types of markers associated with a given project. Data are exportable as spreadsheets or marked-up FASTA files. The marked-up FASTA follows the submission

requirements for design of KBioscience KASP assays and allows bulk design of markers for this technology platform.

Genomic markers stored in KnowPulse are not required to be associated with phenotypes in the same species. If a phenotype-associated marker has not yet been developed for a trait of interest, KnowPulse can be used to find existing genomic markers in regions homologous to a gene thought to be involved in your trait based on studies in other species. A GMOD GBrowse (1) with a *Medicago truncatula* backbone makes it easy to visually inspect a region in *Medicago* for homologous sequences or genomic markers in multiple pulse species.

The cross-species GBrowse is also useful to geneticists due to the ease with which homology among pulse crop species can be visualized. This can help identify potential sequences for gene identification based on information from other species. KnowPulse hosts its own BLAST server (2, 3) with many pulse crop-specific and model plant datasets allowing researchers to find homologous sequences based on sequence similarity. Both of these tools are meant to make it easy to find either sequence or genotypic data based on information from other legume species.

Currently, KnowPulse contains all sequence data generated as part of 454 sequencing-based projects carried out at the CDC in conjunction with the NRC Plant Biotechnology Institute (PBI) in Saskatoon, the *Lens culinaris* EST collection generated previously at CDC/PBI (already deposited to NCBI), all legume DFCI Gene Indices (4) and the latest *Medicago truncatula* assembly (5). All genotypes and markers generated as part of the CDC-led Implementation of Markers for Pulses (iMAP) project are being loaded into KnowPulse. We intend to have most genotypic information generated in our lab publically available through this portal. Any sequence or genotypic information generated by other research groups is welcome and will be included upon request of the originators.

Future development plans for KnowPulse include the ability to store phenotypes including experimental details, environmental conditions and the phenotypes observed for any germplasm surveyed. Once this feature is available, phenotypes can be associated with genotypes, improving the searching capabilities for finding markers associated with a trait of interest. It is also our intention to promote inter-website communication and data-sharing between KnowPulse and other legume-specific web portals including the Legume Information System (6) and the Cool Season Food Legume Genome Database (7).

References

1. Stein L.D et al. The generic genome browser: a building block for model organism system database. 2002. *Genome Research* 12: 1599-1610.
2. Morgulis A et al. Database indexing for production MegaBLAST searches. 2008. *Bioinformatics* 24(16): 1757-1764.
3. Papanicolaou A., Heckel D.G. The GMOD Drupal Bioinformatic Server Framework. 2010. *Bioinformatics* 26(24):3119-3124.
4. Quackenbush J. et al. The TIGR Gene Indices: analysis of gene transcript sequences in highly sampled eukaryotic species. 2001. *Nucleic Acids Research* 29(1): 159-164.
5. Young N.D, et al., The *Medicago* genome provides insight into the evolution of rhizobial symbioses. 2011. *Nature* doi:10.1038/nature10625
6. Gonzales M.D. et al. The Legume Information System (LIS): an integrated information resource for comparative legume biology. 2005. *Nucleic Acids Research* 33(1): D660-D665.
7. Cool Season Food Legume Genome Database: <http://www.gabcsfl.org/>

Progress report on enhancing faba bean (*Vicia faba* L.) germplasm for improved winter-hardiness at Pullman, Washington

Hu, J.^{1,3*}, McGee, R.J.^{2,3},
Landry, E.J.³, Mwangi, J.E.³
and Coyne, C.J.^{1,3}

¹USDA-ARS, PGITU, Pullman, WA USA

²USDA-ARS, GLGRPU, Pullman, WA USA

³Washington State University, Pullman, WA USA

*Presenter

Introduction

Numerous reports have documented that grain legumes have positive effects on the succeeding crop yield (1, 2). Winter-hardy peas and lentils are currently being used in rotation with wheat in the Palouse region of Washington and Idaho. Murray et al. (3) reported that faba bean (*Vicia faba* L.) has the same winter-hardiness as lentil and better than pea. Aiming at developing an alternative rotation crop for cool-temperate regions, we initiated a research project to enhance faba bean germplasm for improved winter-hardiness at Pullman, Washington in 2008. This report summarizes the progress made in the past three years.

Materials and methods

Available faba bean accessions maintained by the USDA-ARS Western Regional Plant Introduction Station in Pullman, WA were used for this project. For the first year experiment we used 43 accessions collected from various countries and 12 winter-hardy cultivars/breeding lines from W. Link of Georg-August University, Germany (4).

Two approaches were taken in screening for winter-hardiness. The first approach is the traditional replicated field trial of single row plots at two locations (Pullman and Central Ferry, WA). Thirty seeds from each entry were planted and observation notes were taken through the growing season. A weather logger was placed near the planting site to collect ambient and soil temperatures through the season. The second approach is a modified "mass selection". Seeds bulked from 466 accessions were planted at a high density in both locations in fall of 2010. Plants that survived through the winter at each location were kept to produce seeds for further testing.

Results and discussion

The first year results were reported earlier (5). We planted 43 accessions in both Pullman and Central Ferry and the 12 winter-hardy lines from Germany were planted in Central Ferry in October 2008. We observed a high level of variation in winter-hardiness among the accessions during the first year. The data loggers recorded minimum ambient temperatures that were at or below -14 °C with at least 5 consecutive nights of hard frost. The number of consecutive days that temperatures stayed below zero ranged from 7 to 14. The extreme was a period in Pullman that reached -22.2 °C and 11 consecutive nights of hard frost. All accessions survived in Central Ferry, while 30 of the 43 accessions were completely killed in Pullman. Accessions that had a higher survival rate in Central Ferry also had a few plants which survived in Pullman. We also observed that some accessions had the ability to send out shoots from the lower nodes of the stems from the plants that were damaged or killed by low temperatures. This ability to "regrow" could be used as one of the criteria to measure winter-hardiness of faba bean.

In October 2009, we planted 75 accessions in both Pullman and Central Ferry. These included 55 accessions that were tested in the first year plus 20 new accessions. Although the winter was not as cold as the previous year, there was little snow to cover the seedlings during the cold months. All the accessions in Pullman were completely dead. Approximately 20 accessions in Central Ferry survived and

the number of plants that survived of each accession varied from one to eleven plants. There was a significant location by year by genotype interaction. Based on the results of survival rate and seed yield potential obtained in the past two years, we selected 16 most winter-hardy accessions for further observation.

In October 2010, the selected accessions were planted in two locations. We also planted nine selected F₃ families derived from a cross between a non-winter hardy vegetable-type variety 'Extra Precoce Vioetto' and a winter-hardy genotype 'Hiverna/2-5EPI' from Germany. All the selected accessions had plants that survived at both locations. Survival rates ranged from 16 – 100%. The plants that survived produced an average of 75g (range 33-150 g) of seeds per plant. All nine F₃ families survived in Central Ferry while only two survived in Pullman. Of these two families, one had only one, and the other had two plants survived. These two families also had a higher survival rate in Central Ferry.

We tried a novel approach of "mass selection" for winter-hardiness. Seeds bulked from 466 accessions were planted at three locations in the Palouse region in October of 2010. The percentage of winter survival was estimated at <1% in Pullman, 5% in Central Ferry and 5-10% in Dayton. Seeds from these plants were harvested for further testing.

In summary, sufficient amount of variation has been captured in the USDA faba bean germplasm collection and satisfactory progress has been made towards establishing a winter-hardy faba bean population in the past three years. These genotypes that survived through the harsh winter in Pullman formed the foundation for developing an alternative fall-sown rotation crop for the Palouse region.

Acknowledgements

Technical assistance from Kristy Ann Ott, Landon Charlo, Wayne Olson, Kurt Tetrick, Sean Vail and Leslie Elberson is gratefully appreciated. Funding includes a Germplasm Evaluation Grant from the USDA Cool Season Food Legume Crop Germplasm Committee to WRPIS, Washington State University and USDA ARS CRIS Project 5348-21 000-026-00D.

References

1. Chalk P.M. 1998. Aust. J. Agric. Res. 4:303–316.
2. Plancquaert P., Desbureaux J. 1985. In: Hebblewithe et al.(ed.) The Pea Crop. Butterworth, London. pp. 193-202.
3. Murray G.A., Eser D., Gusta L.V., Eteve G. 1988. In: Summerfield R.J. (ed.) World Crops: Cool Season Food Legumes. Kluwer, London. pp. 831-843.
4. Arbaoui M., Balko C., Link W. 2008. Field Crops Res. 106:60–67.
5. Hu J., Mwengi J.E., Coyne C.J. and Pan, W.L. 2009. Pisum Genetics. 41:57-58.

Revisiting strategies in lentil breeding: Wild species update

Tullu, A.^{1*}, Bett, K.¹ Saha, S.¹,
Vail, S.² and Vandenberg, A.¹

¹University of Saskatchewan, Saskatoon, SK Canada
²Agriculture and Agri-Food Canada, Saskatoon, SK, Canada

*Presenter (a.tullu@usask.ca)

Canada is the largest exporter and second largest producer of lentils (*L. culinaris* Medikus) in the world and 98% of it is produced in Saskatchewan. When first introduced to Saskatchewan, lentil was grown with relatively fewer problems, however as lentil acreage increased, the crop is now host for many

diseases, such as, anthracnose, ascochyta, stemphyllium, Gray mold, white mold, powdery mildew and root rot complex. A narrow genetic base led to lack of genetic diversity among lentil cultivars. Seven taxa in four species have been reported in lentil gene pool and only *Lens ervoides* species has the highest frequency of resistance followed by *L. nigricans* and *L. lamottei* to both anthracnose and ascochyta diseases. Little or no resistance has been found in the cultivated background for race-Ct0 of anthracnose.

In the breeding program, new lentil cultivars with greater productivity, enhanced quality traits and improved resistance to multiple diseases are becoming increasingly important as a way of maintaining genetic gain in yield. The first breeding approach is to establish interspecific genetic populations to study inheritance pattern and transfer resistance and other key traits to the cultivated background. Recombinant inbred lines (RILs) have been developed using *L. ervoides* and *L. culinaris*, sub sp. *culinaris* crosses. Also, a RIL development between *L. ervoides* accessions is underway. RILs for key traits were selected and backcrossed to adapted backgrounds to transfer favorable genes. Selections derived from the backcross entered into preliminary unreplicated yield trials and results so far are promising.

Another strategy to accelerate utilization favorable genes of wild species is the use of a broad array of genomic and genetic resources. It has been impossible to utilize wild species to improve yield because the superior traits of interest can not be identified phenotypically alone. Additional methods of identifying resistance and key quantitative traits (QTLs) are needed that are more rapid, reliable and useable in earlier generation breeding materials. Significant advances have been made in the development and use of embryo rescue technology, wide hybridizations, library of introgression lines, generating interspecific populations, BAC library, 454-SNPs, COS markers and resistance gene homologues (RGHs).

Developing technology platform for implementing marker assisted selection (MAS) is also another approach in breeding. The amount of introgressed segments can be assessed with SNP genotyping in backcross generations and the introduction of undesirable characteristics such as bushiness, small seededness, etc. during introgression will be minimized using a foreground and background breeding. The goal is to broaden the working germplasm base and increase the genetic gain in yield. As a result, the number of useful new genes is likely to increase and the use of wild species is likely to continue to grow in importance for lentil breeders.

References

1. Vail, S., et al. (2012). Field Crops Research. 126: 145-151.
2. Tullu, A., et al. (2010). Plant Genetic Resources: Characterization and Utilization. 9(01):19-29.
3. Alo, F., et al. (2011). Journal of Heredity. 102 (3): 315-329.

Nitrogen fixation and amino acids in faba-developing a screening tool for improvement

Bueckert, R.A.*, Pritchard, J.
and Vandenberg, A.

University of Saskatchewan, Saskatoon, SK, Canada

*Presenter (rosalind.bueckert@usask.ca)

Pulses can supply most of their N requirements through N₂ fixation, a sustainable means of supplying N to high protein crops. Our goal was to assess 15 genotypes (cultivars and breeding lines of colored flower

types and white flower / low tannin types) of faba (*Vicia faba*) in the field. Faba was grown at two locations in SK in 2009 and 2010, and measured for their ability to take up N and to fix N_2 . The overall goal was to develop a screening method for detecting high N_2 fixation via free amino acids found in leaf, stem and reproductive tissue.

Genotypes fixed N_2 (via a commercial inoculant *Rhizobium leguminosarum* which is marketed to include faba), and the fixed N_2 accounted for 51 to 88% of its requirements depending on environment, the rest coming from root N uptake from the soil. Faba grew more with increasing moisture, but became more indeterminate, and despite higher N accumulation values, yield was not necessarily increased. A range of yield, biomass and N accumulation was seen for Rosthern 2009. Saskatoon 2009 was drier and yields were slightly lower, along with less biomass and slightly less N accumulated. Data for Rosthern for 2010 came from a record wet year, and so biomass was high and maturity was delayed. The 2010 Saskatoon site suffered from waterlogged soil, and despite limited sampling throughout the season, data were not analyzed due to missing plots and unrepresentative plants.

In 2009, Florent, Taboar, Melodie, FB22_10 and Divine had the highest total plant N accumulation, coming from vegetative parts but especially from reproductive growth. The total shoot N content of the 15 genotypes ranged from 32 to 47 g N m⁻². The total shoot nitrogen content can be considered as 8 parts, with 1 part made up of leaf N, less than 1 part made up of stem N, about 1.5 parts being made up of pod shell, and the seed making up the remaining 5 parts. The nitrogen budget, as in the amount in stubble left for succeeding crops, was calculated and was far higher than reported data from Alberta (typical of western Canada), reflecting wet and excellent growing seasons. Additionally, our data are likely to be overestimates due to small plot edge effects. Assuming all N from above ground vegetative biomass at maturity became available to succeeding crops, faba genotypes supplied between 10 and 15 g N m⁻², or 100 to 150 kg N ha⁻¹. The yield portion removed an additional 20 to 35 g N m⁻², or 200 to 350 kg N ha⁻¹, due to faba being a high yielding crop with a high seed protein content (about 30%). For faba to be a good N-supplying crop to a succeeding crop in the rotation, breeding for less yield or lower seed protein concentration would enable more N to be retained in stover.

Despite being an indeterminate crop, faba was efficient at partitioning its above ground biomass to yield with harvest indices ranging from 0.34 to 0.45 in a cool, dry year. For Rosthern in 2009, cultivars had significantly different yields, ranging from 499 to 617 g m⁻² (4,990 to 6,170 kg ha⁻¹). At Saskatoon, yield differences were less obvious among the 15 faba genotypes, ranging from 447 to 650 g m⁻². Snowbird had the highest yield at both locations in 2009. At Rosthern, Snowbird, NPZ5_7680, Divine and Imposa were in the high-yielding group (617-590 g m⁻²). So far, faba's physiological characteristics show a reasonably efficient crop with lower variability when compared to chickpea and lentil (from previous research), but with high amounts of N accumulated in yield and stover. The above ground biomass partitioning of faba was <1 part leaf, >1 to 1.5 parts stem, 1 part pod shell, and the remaining 2 parts seed. The biomass range was 850 to 1670 g m⁻² or 8,500 to 16,700 kg ha⁻¹ in 2009, depending on genotype. Faba is a large biomass crop for an annual crop, likely at the upper end for the prairies.

Faba is classified as a cool season crop like pea and lentil, having amino acid metabolism where the main transport forms of N from N_2 fixation are typically the amides glutamine or asparagine, or amino acids aspartate and glutamate. But faba may have N_2 fixation metabolism in the shoot that appears to be intermediate between cool season pulses and the warm season legumes like common bean (*Phaseolus*) and soybean. Warm season legumes use ureides, which are non-structural cyclic amino acids (allantoin and allantoate), as the major form of N arising from N_2 fixation. Ureides are an efficient means of supplying amino N to the shoot, and may play a role in more stress-tolerant N_2 fixation metabolism, making the crop better suited to dryland production. Ureides may also have a role in stress tolerant N metabolism, aside from N_2 fixation as seen in warm season legumes. Our goal was to measure the ureide concentrations by a colorimetric assay (glyoxylyate production) for leaves, stem and pod material at

flowering, mid pod fill, and close to physiological maturity, and to relate these to plant nitrogen content and yield. Ureides were found in all plant partitions, and appeared to be associated with stress metabolism, as was the amino acid proline, due to consistent negative correlations of these amino acids with N accumulation, fixation and yield. Ureide concentration ranged from 2 to 16 $\mu\text{mol g}^{-1}$ dry tissue, depending on whether leaf, stem or pods were sampled, the environment, and growth stage. The range of ureides seen in faba leaf appear to be greater than in non-droughted chickpea and pea leaf, in stem they are about the same as non-droughted chickpea stem (2 to 5 $\mu\text{mol g}^{-1}$ stem) and less than non-droughted pea stem (3 to 15 $\mu\text{mol g}^{-1}$ stem). The ranges and concentrations of both leaf and stem ureides in faba are lower than soybean leaf (10 to 30 $\mu\text{mol g}^{-1}$ leaf) and petioles (15 to 50 $\mu\text{mol g}^{-1}$).

Free amino acids were also measured by gas chromatography in various plant partitions throughout the lifecycle and correlated against yield, total plant N accumulated and the N_2 fixed. Briefly, faba plants operate mainly with six amino acids, asparagine the major amide, and alanine, serine, tryptophan, cysteine and proline. The major N cycling amino acid is asparagine, followed by leaf metabolism using alanine. Glutamine also plays a role but less is present. Lesser to minimal amounts of aspartic acid and glutamic acid are used. Ureides and proline indicated more stress. We were expecting to find a single amino acid concentration or even a ratio using the amides asparagine or glutamine to aspartate and glutamate to be useful, based on limited studies where amino acid concentrations have been previously published. We found ratios of amino acids more indicative of plant N status than single amino acids, and we plan to use the sum of asparagine plus aspartic acid plus glutamic acid plus glutamine, compared to proline, and a second ratio of alanine to proline, in screening for higher N_2 fixation and N accumulation in faba nurseries. We are currently verifying the amino acid ratios on 2011 plants to test whether this screening tool does find us elite yielding and high N_2 fixing cultivars.

Acknowledgements

Funding from Saskatchewan Pulse Growers Association and the Agriculture Development Fund of Saskatchewan; ureide measurements were done by Ms. Denise Broersma.

***Vicia faba* - a potential rootstock for lentil breeding**

Yuan, H.Y.^{1*}, Lulsdorf, M.¹,
Tullu, A.¹, Gurusamy, V.²
and Vandenberg, A.¹

¹University of Saskatchewan, Saskatoon, SK Canada

²Canadian Wheat Board, Saskatoon, SK Canada

* Presenter(haiying.yuan@usask.ca)

Wild lentil species are an increasingly important genetic resource for lentil breeding programs. An accession of each of the six wild *Lens* species was used as the scion in grafts to faba bean breeding line FB50-9 rootstock. Successful grafts were obtained for all species with survival of grafts to seed maturity between 70.7% and 87.7% except for *Lens orientalis* PI 72735 with 55.3% survival. Flowering time of grafted scions compared to ungrafted controls was not affected for four species but scions of *L. nigricans* PI 72560 and *L. orientalis* PI 72735 had flowering delays of 9 and 7 days respectively. For all six wild species, pod length, seed diameter and seed weight were not significantly different between non-grafted controls and scions grafted onto faba bean rootstocks. This simple approach opens the possibility of using intergeneric *in vivo* grafting techniques to rescue interspecific hybrids of lentil. The technique has potential as a useful tool in lentil breeding, as a means of improving seed multiplication rate of rare genetic resources of wild lentil and as a way to reduce the costs of germplasm multiplication of wild lentil species.

Variety Adaptability and Yield Stability Analysis for a State-wide Variety Testing Study in Montana

Ito, D., Chen, C.* , Neill, K., Hesel, J.,
Eckhoff, J., Kephart, K., Lamb, P.,
Mason, H., McVay, K., Miller, J.,
Miller, P. and Westcott, M.

Montana State University, Moccasin, MT USA

*Presenter (cchen@montana.edu)

Introduction

Dry pea (*Pisum sativum* L.) and lentil (*Lens culinaris* Medik) production in Montana has been rapidly increasing in recent years. Planted acreage of dry pea has been over 200,000 acres since 2006 placing Montana as second largest producer in the nation. Lentil production reached to 260,000 acres in 2010 doubled from the planted acreage in 2009. Pea and lentil fit well in the wheat-based cropping systems as rotation crops to replace fallow, providing many agronomic and economic benefits. However, the most suitable cultivars and agronomic practices have not been well understood in Montana where considerable variation in geography, soil, and the climate exist. Multi-location testing is important to the development and sustainability of the pea and lentil industry in Montana. This paper reports a coordinated statewide dry pea and lentil variety testing from 2008 to 2011. Our objective is to show adaptable varieties with stable yields using additive main effects and multiplicative interactions (AMMI) biplot analysis.

Materials and methods

Selected commercial varieties and breeding lines of dry pea and lentil were planted at nine locations across Montana from 2008 to 2011. Dry pea evaluation consisted of 22 smooth green and yellow cultivars, of which, nine are commercially available, and four are experimental lines from the USDA-ARS Grain Legume Genetics and Physiology program at Pullman, Washington. Lentil evaluation consisted of 13 different classes cultivars, of which, ten are commercially available, and three are experimental lines from the USDA-ARS Grain Legume Genetics and Physiology program. The experiments were randomized complete block design with three to four replications. The grain yield data were subjected to analysis of variance within each year and for combined four years respectively to determine interaction between variety (G) and location (E). Subsequent PCA analysis followed using the same data where each combination between nine locations and four years generating 31 environments for the dry pea variety evaluation and 28 environments for lentil variety evaluation. Genotype plus genotype x environment interaction biplots were generated from the first two principal components (PC1 and PC2). Correlation among the environment was calculated from the angle between two environment vectors.

Results and discussion

Grain yield of both dry pea and lentil varied greatly among the locations across Montana. Significant interactions between variety and location were also observed. The interaction effects (genotype over environment or *vice versa*) can be described as distance from the plot origin. Thus, the most responsive varieties can be visualized in the biplot at the corner or vertex. Majoret, Medora, Stirling, PS0010836, and PS9910140 were identified as extremes. Aragorn, Bridger, Meadow, Patrick and Spider were the most stable varieties among all environments. Among the test sites, Moccasin in all test years was relatively clustered and close to the plot origin indicating less interactive, hence, stable and repeatable location for testing general adaptability in Montana.

The biplot was analyzed for lentil in the same manner as that of dry peas. Brewer, Crimson, Essex, Meteor, Vantage, LC01602245P, and LC01602300R were the most responsive lentil cultivars to the environments while Pennel was the least responsive. Bozeman, Moccasin, and Richland were close to the plot origin indicating these locations were relatively stable. Bozeman and Moccasin were relatively clustered and hence could be good repeatable locations.

Biplot analysis allowed us to identify the most or the least responsive varieties in the various environments. This information is a useful tool to evaluate variety adaptability and yield stability in a particular environment.

Table 1. Dry pea and lentil varieties evaluated in Montana statewide trials, 2008

Pea variety			Lentil variety		
Entry	Variety	Type	Entry	Variety	Type
1	Admiral	Yellow	1	Brewer	Medium Green
2	Aragorn	Green	2	Crimson	Small Red
3	Arcadia	Green	3	Essex	Small Green
4	Bridger	Yellow	4	LC01602062T	Small Red
5	Montech 4152	Yellow	5	LC01602245P	Spanish Brown
6	Cruiser	Green	6	LC01602300R	Medium Green
7	Delta	Yellow	7	Merrit	Large Green
8	Golden	Yellow	8	Meteor	Medium Green
9	K2	Green	9	Pennell	Large Green
10	Majoret	Green	10	Redberry	Small Red
11	Meadow	Yellow	11	Richlea	Medium Green
12	Medora	Green	12	Riveland	Large Green
13	Midas	Yellow	13	Vantage	Medium Green
14	Mozart	Yellow			
15	Patrick	Green			
16	PS0010836	Yellow			
17	PS011102958	Yellow			
18	PS03101822	Yellow			
19	PS9910140	Yellow			
20	Spider	Yellow			
21	Stirling	Green			
22	Striker	Green			

Characterization of mycosphaerella blight resistance, lodging resistance, and micronutrient concentration in a pea recombinant inbred line population

Liu, Y.^{1*}, Tar'an, B.¹, Banniza, S.¹,
Sharpe, A.² and Warkentin, T.¹

¹University of Saskatchewan, Saskatoon, SK, Canada

²NRC-PBI Saskatoon, SK, Canada

*Presenter (yol828@mail.usask.ca)

Abstract

Moderate variation for adult plant resistance to mycosphaerella blight, pre-harvest lodging and selenium accumulation have been observed in field pea. In order to map the quantitative trait loci (QTLs)

associated with these traits, a population of 142 F_9 and F_{10} recombinant inbred lines (RILs) derived from a cross between Carrera (susceptible to mycosphaerella blight and lodging) and CDC Striker (moderately resistant to mycosphaerella blight and lodging) were phenotyped in Saskatoon and Rosthern, SK, Canada in 2010 and 2011. Based on phenotypic data collected from 2010 and 2011, area under the disease progress curve (AUDPC) of mycosphaerella blight ranged from 131 to 205 and 144 to 235 for Saskatoon and Rosthern, respectively. At physiological maturity, lodging ratings of the RILs ranged from 3.8 to 8.3 at Saskatoon and 4.5 to 8.5 at Rosthern. Micronutrient (Se, Zn and Fe) concentrations in the seeds of each RIL were determined using an atomic absorption spectrophotometer for the 2010 samples. A genetic linkage map was generated using 65 SSR (microsatellite) markers resulting in 13 linkage groups that cover 290.3cM of the pea genome. A region between AA491 and AA278 on linkage group 13 was identified as putative QTLs associated with mycosphaerella blight, lodging, Zn and Fe concentrations. All QTLs were derived from CDC Striker, except the one associated with higher Zn concentration which was derived from Carrera.

Introduction and objective

Field pea production in western Canada is negatively affected by several fungal diseases and lodging. The major fungal disease is mycosphaerella blight. Complete resistance to mycosphaerella blight is lacking in field pea germplasm. Pea cultivars with weak stems show severe lodging after flowering, causing reductions in forage and seed yield (Stelling, 1994). Lodging resistance enhances harvest and is associated with reduced severity of mycosphaerella blight (Banniza et al. 2005). Soils in Saskatchewan are rich in Se. Field pea cultivars grown in Saskatchewan displayed a moderate level of variation in Se accumulation (Thavarajah et al. 2010), whereas large regions of Asia and Europe have soils deficient in Se (Gawalko et al. 2009), thus a good opportunity is available to market Canadian peas to these regions for nutritional benefits. The objective of this study is to determine the genetic control of several traits in field pea including mycosphaerella blight resistance, lodging resistance and selenium concentration by genotyping and phenotyping a recombinant inbred line population segregating for these traits.

Methods

A pea population consisting of 142 RILs was developed from a cross between Carrera and CDC Striker. In 2010 and 2011 field trials, pea seeds were planted in microplots (1 m²) with two replications in each of two locations (Saskatoon and Rosthern, SK). Fertility and management practices were provided sufficient for pea production in these regions. From two weeks after flowering until maturity, assessments were made to record mycosphaerella blight severity and lodging. Mycosphaerella blight was rated under natural infection on the basis of all plants in a plot using 0-9 scale where 0 = no disease and 9 = whole plant severely blighted. Four assessments of mycosphaerella blight were done and calculated into area under disease progress curve (AUDPC). Lodging was assessed 4 times during the season and one time at physiological maturity on a 1-9 scale where 1 = completely upright and 9 = completely lodged.

Regarding genotyping, young fresh leaves were randomly sampled from 5 plants from each RIL grown in a greenhouse for genomic DNA extraction by hexadecyltrimethyl ammonium bromide (CTAB) method. A total of 330 Simple Sequence Repeat (SSR) primers derived from the AgroGene consortium (France) were screened on the parents using polyacrylamide gel electrophoresis (PAGE). Polymorphic markers between parents were screened among RILs by using an Applied Biosystem 3730 DNA analyzer.

Results and discussion

Significant effects of genotype, location and year were detected for AUDPC (mycosphaerella blight) and lodging in 2010 and 2011. At Saskatoon, AUDPC ranged from 131 to 205 among RILs, while at Rosthern it ranged from 144 to 235. At physiological maturity, lodging ratings of the RILs ranged from 3.8 to 8.3 at Saskatoon and 4.5 to 8.5 at Rosthern. Lodging was significantly correlated with mycosphaerella blight score ($r = 0.35$; $P < 0.001$), which suggests that RILs that are less susceptible to lodging may escape disease to some extent. A total of 112 out of 330 SSR markers were polymorphic between Carrera and CDC

Striker and 65 out of 112 SSRs were polymorphic among the RILs. A total of 13 linkage groups were generated consisting of 56 SSRs with 9 unlinked by using Carthagene 1.2.2 (De Keyser et al. 2010). QTL analysis was conducted by composite interval mapping (CIM) using the Qgene program to detect associations between phenotype and genotype. A region between AA491 and AA278 on linkage group 13 was identified as putative QTLs associated with mycosphaerella blight, lodging, Zn and Fe concentrations. All QTLs were derived from CDC Striker, except the one associated with higher Zn concentration which was derived from Carrera. Phenotypic variation explained by these QTLs associated with mycosphaerella blight resistance, lodging resistance, Zn and Fe concentrations were 28.5%, 10.1%, 7.4% and 13.1%, respectively.

Acknowledgments

We would like to thank Ms. Reshma Rizvi, Mr. Brent Barlow, Dr. Aziz Rehman, and Ms. Carmen Breitzkreutz for their great help during this project. The technical support from the field lab crew was also appreciated. Funding for this research was supplied by Saskatchewan Pulse Growers and Saskatchewan Ministry of Agriculture.

References

1. Banniza, S., Hashemi, P., Warkentin, T.D., Vandenberg, A. and Davis, A.R. 2005. The relationships among lodging, stem anatomy, degree of lignification and resistance to mycosphaerella blight in field pea (*Pisum sativum*). Can. J. Bot. 83: 954-967.
2. De Keyser, E. D., Shu, Q. Y., Bockstaele, E.V., and Riek, J.D. 2010. Multipoint-likelihood maximization mapping on 4 segregating populations to achieve an integrated framework map for QTL analysis in pot azalea (*Rhododendron simsii* hybrids). BMC MolBiol, 11 (1): 1-20.
3. Gawalko, E., Garrett, R.G., Warkentin, T., Wang, N., and Richter, A. 2009. Trace elements in Canadian field peas: a grain safety assurance perspective. Food Additives and Contaminants. 26 (7): 1002-1012.
4. Joehanes, R., and Nelson, J.C. 2008. Qgene 4.0, an extensible Java QTL-analysis platform. Bioinformatics Applications Note, 24: 2788-2789.
5. Semagn, K., Bjornstad, A., and Ndjiondjop, M.N. 2006. An overview of molecular marker methods for plants. African Journal of Biotechnology. 5 (25): 2540-2568.
6. Thavarajah, D., Warkentin, T. and Vandenberg, A. 2010. Natural enrichment of selenium in Saskatchewan field peas (*Pisum sativum* L.). Can. J. Plant Sci. 90: 383-389.

Comparison of transcriptomes between *Sclerotinia sclerotiorum* and *S. trifoliorum* using 454 Titanium RNA sequencing

Qiu, D.¹, Vandemark, G.² and Chen, W.^{2*}

¹Washington State University, Pullman, WA, USA

²USDA-ARS, Pullman, WA, USA

*Presenter

Both *Sclerotinia sclerotiorum* and *S. trifoliorum* cause Sclerotinia stem and crown rot of chickpea and white mold on many economically important crops. The host range of *S. trifoliorum* is mainly on cool season forage and grain legumes of about 40 plant species, whereas the host range of *S. sclerotiorum* encompasses more than 400 plant species including all the host plant species of *S. trifoliorum*. Despite of morphological and ecological differences between the two species, both species are equally pathogenic on chickpea. Extensive research has been conducted on *S. sclerotiorum* and its genome sequences are available. However, relatively very little is known about *S. trifoliorum*. To take advantages of the genomic

information of *S. sclerotiorum*, we compared the transcriptome of *S. trifoliorum* with that of *S. sclerotiorum* in order to gain a better understanding of the biology of both species. Total mRNAs of both species during vegetative growth were extracted and sequenced using the latest 454 Titanium RNA sequencing technology. A total of 23325 unique transcripts with average length of 534 nt (12.5 mb genome coverage) were obtained from *S. sclerotiorum*, whereas 21214 unique transcripts with average length of 509 nt (10.8 mb genome coverage) were obtained from *S. trifoliorum*. Comparison of the transcripts between the two species will be presented and their implications will be discussed.

Insertional mutation at the Cu-Zn-superoxide dismutase gene reduces virulence of *Sclerotinia sclerotiorum* on pea (*Pisum sativum*)

Xu, L.¹ and Chen, W.^{2*}

¹Washington State University, Pullman, WA, USA

²USDA-ARS, Pullman, WA, USA

*Presenter

Sclerotinia sclerotiorum causes white mold disease on pea and on many other economically important pulse, vegetable and field crops, demonstrating a non-host-specific pathogenic mechanism. Despite extensive studies on this pathogen, its pathogenic mechanisms are still incompletely understood. In order to gain insight in understanding its non-specific host-pathogen interactions, Agrobacterium-mediated transformation (AMT) was used to generate random mutations and to identify potential virulence/pathogenicity factors in *S. sclerotiorum*. Among several hundreds of AMT transformants screened, two stable mutants showed significantly less virulence in comparison with the wild type strain as measured by colonizing pea leaves in detached leaf assays. Southern hybridization and inverse PCR analyses showed that the mutation was due to a single T-DNA insertion at the gene Cu-Zn-superoxide dismutase (SsSOD1, SSIG_00699) of *S. sclerotiorum*. In addition to reduced virulence, the mutant had reduced tolerance to heavy metal toxicity and oxidative stress. The SsSOD1 gene was able to functionally complement SOD in a yeast strain defective of the SOD gene. There was more accumulation of superoxide in disease lesions caused by the mutant than that caused by the wild type strain. Evidence showed that the SsSOD1 gene is an important virulence factor of *S. sclerotiorum*.

Rhizoctonia root rot of lentil caused by *Rhizoctonia solani* AG 2-1

Paulitz, T.C.¹, Schroeder, K.L.¹
and Chen, W.^{2*}

¹USDA-ARS-RDBCUC, Pullman, WA, USA

²USDA-ARS-GLGPRU, Pullman, WA, USA

*Presenter

Lentil root rot symptoms were observed in commercial fields in the US Pacific Northwest during the unusually cool and moist spring weather of 2010. Symptoms included sunken lesions on root and stem with brown discoloration, resembling diseases caused by *Rhizoctonia solani*. *Rhizoctonia solani* was isolated from diseased plants and from surrounding soils and were identified to be AG 2-1 based on ITS sequences. Pathogenicity tests were conducted at 16C 12 h day and 10 C night temperature conditions using three isolates of *R. solani* AG 2-1 from lentil. Isolates were severely pathogenic to lentil cvs. Pardina

and Merrit, as well as to spring canola cv Sunrise, and the pathogen was reisolated from the inoculated plants. *Rhizoctonia solani* AG 2-1 is known to be a pathogen of canola in Australia, Canada and US Pacific Northwest, but not previously isolated from lentil. This is the first report of *Rhizoctonia solani* AG 2-1 infecting lentil.

Field evaluation of fungicides for control of ascochyta blight of chickpeas in North Dakota

Wunsch, M.J.^{1*}, Bradbury, G.²,
Schaeffer, M.¹ and Schatz, B.G.¹

¹Carrington Research Extension Center, Carrington, ND USA

²Williston Research Extension Center, Williston, ND USA

*Presenter (michael.wunsch@ndsu.edu)

Registered and experimental fungicides, including several products with anticipated registration in 2012 and 2013, were evaluated for their control of ascochyta blight caused by *Ascochyta rabiei* in Carrington, Minot, and Williston, ND in 2011. Significant differences in fungicide efficacy were observed among products, and at least one experimental chemistry gave excellent disease control. Disease efficacy, seed yield, and seed quality results will be reported.

Field evaluation of fungicides for control of anthracnose, botrytis gray mold, and sclerotinia stem rot of lentil in North Dakota

Wunsch, M.J.^{1*}, Pederson, J.²,
Schaeffer, M.¹ and Schatz, B.G.¹

¹Carrington Research Extension Center, Carrington, ND USA

²North Central Research Extension Center, Minot, ND USA

*Presenter (michael.wunsch@ndsu.edu)

Registered and experimental fungicides, including several products with anticipated registration in 2012 and 2013, were evaluated for their control of anthracnose, caused by *Colletotrichum truncatum*, and sclerotinia stem rot, caused by *Sclerotinia sclerotiorum*, in Carrington and Williston, ND in 2011. Fungicide timing was also evaluated in Carrington and Minot, ND for the control of anthracnose and botrytis gray mold, caused by *Botrytis cinerea*. Significant differences were observed among treatments; disease efficacy, seed yield, and seed quality results will be reported.

Field evaluation of fungicides for control of ascochyta and mycosphaerella blights of field peas in North Dakota

Wunsch, M.J.^{*}, Schaeffer, M.
and Schatz, B.G.

Carrington Research Extension Center, Carrington, ND USA

^{*}Presenter (michael.wunsch@ndsu.edu)

Registered and experimental fungicides, including several products with anticipated registration in 2012 and 2013, were evaluated for their control of Ascochyta and Mycosphaerella blights caused by *Ascochyta pisi*, *A. pinodes*, and/or *Phoma pinodella* in Carrington and Newburg, ND in 2010 and 2011. Significant differences in fungicide efficacy were observed among products, and several experimental chemistries gave excellent disease control. Disease efficacy, seed yield, and seed quality results will be reported.

Natural outcrossing rate of faba bean under Pullman field conditions and its implication to germplasm management and enhancement

Hu, J.^{1,2*}, Landry, E.J.²,
Mwengi, J.E.² and Coyne, C.J.^{1,2}

¹USDA-ARS, WRPIS, Pullman, WA USA

²Washington State University, Pullman, WA USA

^{*}Presenter

Abstract

Knowledge of the natural outcrossing rate of faba bean (*Vicia faba* L.) under Pullman field conditions will enable us to refine strategies for both germplasm management and enhancement. We used the white flower phenotype governed by a recessive gene in the investigation of the natural outcrossing rate of faba bean. Seeds from white flowered plants grown in Pullman, 2010 were harvested and planted plant-to-row in spring 2011. During flowering, the number of plants with white or regular colored flowers was recorded for each row. The percentage of plants with regular colored flowers was used as an estimate of natural outcrossing rate, which averaged 30.8% with a range from 0 to 82.6 % among 50 rows. This observed outcrossing rate is within the range of previous reports for faba bean grown in various locations. The high outcrossing rate is likely the result of abundant bumble bees and honey bees which visited the faba bean flowers frequently during bloom. Therefore, for germplasm management it is necessary to regenerate faba bean accessions using insect-proof cages to maintain the genetic integrity of individual accessions. For germplasm enhancement using phenotypic selection, it is also crucial to physically isolate the selected plants with insect-proof bags to prevent unwanted cross-pollinations and produce self-pollinated seeds for subsequent generations.

Introduction

Faba bean (*Vicia faba* L.) is a partially allogamous crop and the outcrossing rate has been investigated by many researchers in various locations. These research results reported prior to 1980s were summarized by Bond and Poulsen (1) and ranged 4 to 84%. More recent reports were within this range (2; 3 and 4). It has been reported that the outcrossing rate for faba bean is strongly influenced by local environmental and climate conditions as well as the presence of pollinating insects such as species of bumble-bees, honey bees, and solitary bees.

The U.S. National Plant Germplasm System (NPGS) is one of the world's largest national genebank networks focusing on preserving the genetic resources of crops and wild relative species for the continuing improvement of agricultural productivity. The USDA faba bean germplasm collection is maintained by the Western Regional Plant Introduction Station (WRPIS) in Pullman, Washington. Knowledge of the natural outcrossing rate of this crop under Pullman field conditions will enable us to refine strategies for both germplasm management and enhancement.

Materials and methods

Plants with pure white flowers were found in 13 among 466 accessions that were planted in 2010 for evaluation. Two of the 13 accessions consisted of only white flowered plants while the remaining 11 accessions were segregating. Open-pollinated seeds from these white flowered plants were harvested in fall 2010 and planted plant-to-row in spring 2011. During flowering, plants with white and plants with regular colored flowers were counted and recorded for each row. The percentage of plants with regular colored flowers was calculated with the formula of (number of plants with regular colored flower/total number of plants)*100 and used as an estimate of natural outcrossing rate.

Results and discussion

There were a total of 810 plants scored for flower color in the 50 rows, each of which was derived from the open-pollinated seeds of a single white-flowered plant grown in Pullman in 2010. The number of plants per row varied from two to 25. A wide range of outcrossing rate from 0 to 82.6 % was observed among the 50 rows. The average was 30.8% and the standard error was 2.98 (Figure 1). The high outcrossing rate is likely the result of abundant bumble bees and honey bees which visited the faba bean flowers

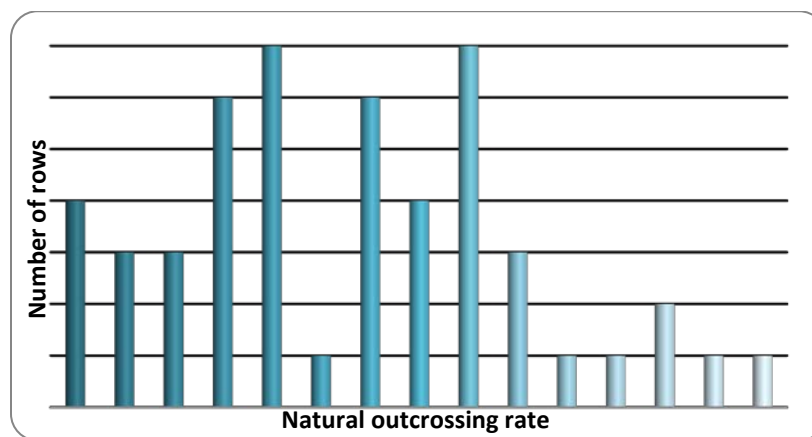


Figure 1. Wide range of natural outcrossing rates observed among the 50 single-plant-derived rows.

frequently during bloom. Therefore, for germplasm management it is necessary to regenerate faba bean accessions using insect-proof cages to maintain the genetic integrity of individual accessions. For germplasm enhancement using phenotypic selection, it is also crucial to physically isolate the selected plants with insect-proof bags to prevent unwanted cross-pollinations and produce self-pollinated seeds for subsequent generations.

Acknowledgements

Assistance from Landon Charlo, Wayne Olson, Kurt Tetrick, Sean Vail and Leslie Elbertson is gratefully appreciated. Funding includes a Germplasm Evaluation grant from the USDA Cool Season Food Legume Crop Germplasm Committee and the USDA-ARS CRIS Project 5348-21 000-026-00D.

References

1. Bond, D. A., Poulsen, M. H. 1983. In: Hebblethwaite PD, editor. The Faba Bean (*Vicia faba* L.). Butterworths, London , pp. 77–101.
2. Cartujo, F., Suzo, M. J., Pierre, J., Moreno, M. T., Le Guen, J. 1998. Proc. International Symposium on Breeding of Protein and Oil crops. Eucarpia, Pontevedra, Spain. p. 49-50
3. Suso, M. J., Moreno, M. T. 1999. Plant Breeding 118:347–350.
4. Suso, M. J., Pierre J., Moreno, M.T., Esnault, R. and Le Guen, J. 2001. J. Agric. Sci. Camb. 136: 399–405.

Genome mapping and molecular markers for Ascochyta Blight resistance in pea (*Pisum sativum* L.)

Miranda, A.* and McPhee, K.

North Dakota State University, Fargo, ND USA

*Presenter (andre.miranda@ndsu.edu)

Ascochyta blight is the most common disease of economic importance in peas (*Pisum sativum* L.) in North Dakota. It is caused by three pathogens: *Ascochyta pisi* Lib., and *Mycosphaerella pinodes* (Berk & Bloxam) Vesterg., which causes leaf and pod spot; and *Ascochyta pinodella*, recently designated *Phoma medicaginis* var. *pinodella* (L. K. Jones) Boerema, which causes foot rot. The ultimate goal of this research is to reduce the economic impact of Ascochyta blight on the pea crop. Objectives of this research are: 1) to implement marker-assisted selection (MAS) in the breeding program to enhance plant selection for cultivar development, 2) to develop pea varieties that are resistant to the Ascochyta blight that occurs in ND, and 3) integration of field and laboratory technologies to provide sustainable disease management that is compatible with overall crop production in the region. An F₇-derived recombinant inbred line population of 394 lines was developed from the cross 'Lifter'/'Radley' and DNA was extracted from each RIL for PCR-based marker analysis. Phenotypic evaluations were conducted in the greenhouse during the summer of 2011. Five replicate plants were scored using 0 to 5 scale, where 0 = no disease and 5 = high incidence of disease or plant death. Disease ratings were taken on 5 different dates, starting 21 days after planting and every 3 days subsequently. Forty-three lines showed a high level of resistance and RIL-369 and RIL-387 showed the greatest level of resistance. QTL analysis was conducted to identify DNA markers associated with Ascochyta blight resistance genes in the Lifter/Radley population that can be applied in the pea breeding program.

Developing a method to scale up production of solanapyrone toxins by *Ascochyta rabiei*

Kim, W.¹, Vandemark, G.²
and Chen, W.^{2*}

¹Department of Plant Pathology, WSU, Pullman, WA, USA

²USDA-ARS, Pullman, WA, USA

*Presenter

Ascochyta rabiei, the causal agent of Ascochyta blight of chickpea, produces solanapyrone toxins. The toxins may play a role in pathogenesis, and a toxin assay using chickpea plant tissues may enable

screening breeding materials at early generations for resistance to blight. In order to develop toxin assays for screening chickpea genotypes and to investigate the role of the toxins in developing the disease *Ascochyta* blight, sufficient quantity of the toxins is needed for replicated and repeated experiments. A new method with solid medium using natural substrates is being developed to replace the previously published method with liquid medium in static culture. The new method significantly improved production in larger quantity of the solanapyrone toxins with much reduced requirement of laboratory space, and will facilitate future research employing and exploiting the solanapyrone toxins.

First report of stemphylium blight of lentil in Montana and North Dakota: evidence, distribution, and prospects for management.

Wunsch, M.J.*

Carrington Research Extension Center, Carrington, ND USA

*Presenter (michael.wunsch@ndsu.edu)

Stemphylium blight, caused by the fungal pathogen *Stemphylium botryosum*, was observed for the first time in Montana and North Dakota in 2010 and 2011. The disease occurred in all production regions surveyed, sometimes at moderate to high severity. Stemphylium blight severity data collected in a fungicide timing trial and variety performance trials suggest that commonly employed fungicide application strategies may not be effective against the disease but that several lentil varieties adapted to the region may show some resistance.

Response of chickpea varieties to foliar fungicides

Pederson, S.¹* and McPhee, K.²

¹NCREC, North Dakota State University, Minot, ND USA

²North Dakota State University, Fargo, ND USA

*Presenter (shana.pederson@ndsu.edu)

Ascochyta blight (*Ascochyta rabiei*) can be devastating to chickpea (*Cicer arietinum* L.) production in North Dakota. *Ascochyta* susceptible varieties, as well as resistance in the pathogen to strobilurin fungicides, have impacted production. In order to determine an effective screening method for the chickpea breeding program, an evaluation of the response of varieties from different market classes to foliar fungicides was essential. Eight chickpea varieties were grown at four North Dakota locations. Agronomic performance and quality of each variety was compared in a fungicide/no fungicide management system. In 2010, seed yield, canopy height, 1000KWT, disease ratings and seed sizing between fungicide treatment and the untreated control at two locations were significant. Preliminary results from 2011 indicate a similar trend. Significant yield differences of each variety in response to foliar fungicide were more evident at higher yielding locations. Other production constraints may have impacted this response at lower yielding locations. Based on two years of evaluations, it is clear that fungicide treatment of breeding materials is necessary in North Dakota environments to ensure seed production and prevent loss of valuable germplasm. Even in the presence of fungicide treatment, significant disease is evident and sufficient to allow differential selection among germplasm lines. This research will allow breeding programs to

effectively manage Ascochyta blight to an advantage and avoid complete loss and setbacks in breeding and selection of improved varieties.

Pea germplasm with partial resistance to *sclerotinia sclerotiorum* that extends the time required by the pathogen to infect host tissue

Porter, L.D.*

USDA-ARS, VFCRU, Prosser, WA, USA

*Presenter (Lyndon.porter@ars.usda.gov)

White mold, caused by the fungus *S. sclerotiorum* can be a serious disease on pea. Currently there are no pea genotypes with complete resistance to this pathogen. Selected wild pea genotypes from the *Pisum* Core Collection and cultivars were assessed for the time required by *S. sclerotiorum* to severely infect these genotypes at all combinations of five temperatures (15.6, 18.3, 21.1, 23.9, 29.4°C) and four (12, 24, 48 and 72 h) periods of high relative humidity (PHRH). Commercial genotypes did not prevent severe infection at any temperature/PHRH combination. Three wild pea genotypes were capable of preventing severe infection for up to 24 hours. PII69603 and PI240515 are recommended to pea breeders as the best germplasm to extend the time required for serious infection by *S. sclerotiorum*.

Use of resistant-susceptible cultivar mixture to preserve *er-1* gene rendering resistance to powdery mildew of field pea (*pisum sativum* L.)

Bing, D.J.^{1*}, Gan, Y.²
and Warkentin, T.³

¹AAFC, Lacombe Research Centre, Lacombe, AB Canada

²AAFC, Semiarid Prairie Agricultural Research Centre, Swift Current, SK, Canada

³CDC, University of Saskatchewan, Saskatoon, SK, Canada

*Presenter (dengjin.bing@agr.gc.ca)

Powdery mildew (caused by *Erysiphe pisi* DC. var *pisi*.) resistance of field pea (*Pisum sativum* L.) cultivars is rendered by a recessive gene *er-1*. Cultivation of resistant cultivars over large geographic areas creates a typical gene monoculture, which encourages the pathogen evolution for more virulent races and breakdown of the resistance. We investigated the use of mixtures made up of resistant and susceptible cultivars to limit the pathogen evolution and to preserve the resistance gene. Since severe powdery mildew infection can cause significant yield reduction, it is necessary to define a proper ratio for such cultivar mixtures. Seed of three powdery mildew resistant cultivars was mixed with 10, 20, or 30% of a susceptible cultivar, and grown in replicated field trials. The yield reduction in cultivar mixtures depended on the yield potential and performance of component cultivars and disease severity. When disease severity was high and the resistant cultivar yielded well, the susceptible cultivar could comprise 10-30% of the mixture without significant yield reduction compared with the resistant cultivars in pure stand. The results suggest that such cultivar mixtures may be used in field pea production by providing more substrate to the pathogen so that the breakdown of resistance gene *er-1* may be delayed.

Powdery mildew is a common disease of field pea, and can cause significant yield losses. The majority of powdery mildew resistant field pea cultivars carry a single recessive gene *erl*. Cultivation of such resistant cultivars over large geographic areas could press the pathogen to mutate for more virulent race(s), accompanied by a sudden breakdown of the resistance. We propose to use cultivar mixtures consisting of resistant and susceptible cultivars in an appropriate ratio to provide more substrate to the pathogen, and therefore to reduce selection pressure on the pathogen, so that the life time of the *erl*-rendered resistance may be extended. The main objective of this study was to define proper ratios of resistant-susceptible cultivar mixtures with no or little yield reduction compared to the resistant cultivars in pure stands.

Materials and methods

Three powdery mildew resistant field pea cultivars, Agassiz, Cutlass, and Reward were mixed with the powdery mildew susceptible cultivar, CDC Striker, in 10, 20 or 30% by number of seeds to make resistant-susceptible cultivar mixtures. Such mixtures were grown in replicated field trials along with the four cultivars in pure stands. Also included in the study was CDC Striker sprayed with the fungicide Kumulus (denoted as CDC Striker + Kumulus), where powdery mildew was controlled by spraying Kumulus on the plants when the disease symptoms occurred on the plants. The seeding rate was adjusted to 85 viable seeds m⁻². The yield difference between CDC Striker+Kumulus and CDC Striker was the measure of yield reduction caused by powdery mildew, and the yield of each cultivar in pure stand was compared with the yield of each of its mixtures with CDC Striker to determine the yield reduction of each cultivar mixture. The experimental design was a randomized complete block with four replications. The field trials were grown in four locations: Lacombe, AB; Saskatoon and Swift Current, SK; and Morden, MB in 2006 and 2007. However, the disease severity at other three locations in both years was much less than the disease severity in Lacombe, which may lead to biased estimations of the impact of the disease on the yield. Therefore, only the results from Lacombe were reported here.

Results and discussion

Yield reduction caused by powdery mildew.

CDC Striker+Kumulus yielded 3766 kg ha⁻¹ in 2006 and 3760 kg ha⁻¹ in 2007. In comparison, CDC Striker yield 3033 kg ha⁻¹ and 2483 kg ha⁻¹ in 2006 and 2007, respectively. Thus, CDS Striker yielded 24% and 32% less than CDC Striker+Kumulus. This yield reduction was attributed to the powdery mildew infection. The higher yield reduction in 2007 than in 2006 was attributed to the higher disease severity in 2007. This signifies the importance to preserve the resistance conferred by gene *erl*.

Proper ratio of resistant vs. susceptible cultivar in a cultivar mixture. No significant

Table 1. Yield difference of cultivars in pure stands and cultivar mixtures		
	Year	Difference ^a
Agassiz – (Agassiz70+CDC Striker30)	2006	130
	2007	799 (p=0.053)
Agassiz – (Agassiz80+CDC Striker20)	2006	-521
	2007	956 *
Agassiz – (Agassiz90+CDC Striker10)	2006	-223
	2007	440
Cutlass – (Cutlass70+CDC Striker30)	2006	34
	2007	994 *
Cutlass – (Cutlass80+CDC Striker20)	2006	-86
	2007	501
Cutlass – (Cutlass90+CDC Striker10)	2006	-266
	2007	330
Reward – (Reward70+CDC Striker30)	2006	460
	2007	-246
Reward – (Reward80+CDC Striker20)	2006	-226
	2007	-53
Reward – (Reward90+CDC Striker10)	2006	-92
	2007	-122

^aYield difference (kg ha⁻¹) between resistant cultivar in pure stands and cultivar mixture. * = p < 0.05.

difference was found between any of the three cultivars in pure stands and their mixtures with CDC Striker in 2006 (Table 1), whereas in 2007, Agassiz had significantly higher yield than Agassiz80+CDC Striker20, and nearly significant higher yield than Agassiz70+CDC Striker30. Cutlass had significantly higher yield than Cutlass70 + CDC Striker30 in 2007. There was no significant difference between Reward and its mixtures with CDC Striker. The difference between 2006 and 2007 was attributed to the combination of two factors. In general yield and powdery mildew severity were lower in 2006 than in 2007. Thus, the yield potential of the resistant cultivars was not fully expressed in 2006, and the yield reduction caused by powdery mildew was less in 2006 than in 2007.

Conclusion

The proper ratio of resistant vs. susceptible cultivar in a cultivar mixture depends on yield potential of component cultivars and powdery mildew severity. When disease severity was high and the resistant cultivar yielded well, the susceptible cultivar could comprise 10-30% of the mixture without significant yield reduction compared with the resistant cultivar in pure stand. Such cultivar mixtures may be used in field pea production by providing more substrate to the pathogen so that the breakdown of resistance gene *erl* may be delayed.

Variability for micronutrient composition of pea and lentil grown in Montana

McPhee, K.^{1*}, Thavarajah, D.²,
Thavarajah, P.¹ and Chen, C.³

¹North Dakota State University, Dept. 7670, Fargo, ND USA

²North Dakota State University, Dept. 7640, Fargo, ND USA

³Montana State University, CARC, Moccasin, MT USA

*Presenter (kevin.mcphee@ndsu.edu)

Pulse crop production in North Dakota and the Midwest region including eastern Montana and South Dakota has increased over the past 15 years to become the primary production region for these crops in the US. Pea and lentil are well regarded as highly nutritious foods and serve as the primary protein source in diets for many populations worldwide. In addition to serving as an excellent source of protein, starch and fiber, pea and lentil seed contain many additional micronutrients that contribute to the daily nutritional requirements. This research was conducted to explore the environmental effects on micronutrient accumulation in pea and lentil and to provide a base line for future variety development. Seed harvested from genotypes comprising the statewide yield trials conducted at eight locations in Montana were analyzed for micronutrient composition using high performance liquid chromatography (HPLC) and inductively coupled plasma emission spectroscopy (ICP-Emission) methodology. Iron (Fe) concentration ranged from 46.3 to 68.0 mg/kg among 21 lentil genotypes and from 50.5 to 69.1 mg/kg across the eight locations. Fe concentration in pea ranged from 37.3 to 51.2 mg/kg among 27 genotypes and from 38.2 to 50.5 mg/kg across nine locations. Zinc (Zn) concentration ranged from 31.2 to 43.7 mg/kg among 21 lentil genotypes and from 27.6 to 50.0 mg/kg across eight locations. Zn content among 27 pea genotypes ranged from 31.9 to 129.0 mg/kg and from 29.4 to 124.1 mg/kg across nine locations. Additional micronutrients and antinutrients including carotenoids and phytic acids were also analyzed. Proximity of locations appeared to show a trend in seed micronutrient content. These data indicate that environmental factors, i.e. soil type, may have a significant role in accumulation of micronutrients in pea and lentil. This research serves as the basis for future variety development focused on increasing the micronutrient composition of pea and lentil seed with intent of providing greater nutrition to undernourished populations worldwide.

Chemical composition and mineral content of flours from various varieties of mature and immature yellow pea.

Wang, N.* and Gawalko, E.

Grain Research Laboratory, CGC, Winnipeg, AB, CANADA

*Presenter (ning.wang@grainscanada.gc.ca)

The present study was undertaken to investigate how immature seeds of yellow pea affect chemical composition and mineral content of flours from various varieties of yellow peas. Three yellow pea varieties were used in this study. Mature and immature seeds in the samples were manually picked, dehulled and then ground into flours. Chemical composition, sugar and mineral content of the pea flours were analysed according to published methods. Preliminary results demonstrated that immature seeds displayed a significant effect on chemical composition, sugar and mineral content of flours from yellow peas.

Pisum Genetics Association Membership List

Agriculture & Agri-Food Canada
Research Centre - Library
101 Route 100 - Unit 100
MRODEN - MB R6M1Y5
CANADA

Mike Ambrose
Dept. of Crop Genetics
John Innes Centre
Colney Lane
Norwich NR4 7UH, UK
mike.ambrose@bbsrc.ac.uk

Amelioration des Plantes &
Biotechnologies Vegetales
Domaine de La Motte
BP 35327
35653 Le Rheu CEDEX, France

Bob Arthur
Crites-Moscow Growers, Inc.
P.O.Box 8912
Moscow, ID 83843-1412, USA
Tel: 208-882-5519
Bob@critesmoscow.com

Dr. V.S. Bogdanova
Inst. of Cytology and Genetics
Lavrentiev Ave. 10
Novosibirsk 630090, Russia
vera@bionet.nsc.ru

Dr. Alex Y. Borisov
Res. Inst. for Agric. Microbiology
Podbelsky Shosse 3
St.Petersburg-Pushkin 8, 189 620
Russia
rifam@mail.rcom.ru

Abel J. Bouwman
Limagrain Nederland BV
P.O. Box 1
4410 AA Rilland
The Netherlands
abel-jan.bouwman@limagrain.com

British Library, Acquisitions Unit
Boston Spa
Wetherby, West Yorks, LS23 7BQ
England

Bulgarian Academy of Sciences
Central Library
1, 7 Noemvri Street
1040 Sofia, Bulgaria

Steven Cannon
1017 CGIL
Iowa State University
Ames, IA 50011, USA
Tel: 515-294-6971
scannon@iastate.edu

Cornell University
Acquisitions Division
Albert Mann Library
Ithaca, NY 14853-4301

Dr. Clarice J. Coyne
USDA-ARS, WRPIS
59 Johnson Hall
Washington State University
Pullman, WA 99164-6402, USA
Tel: 509-334-3227
coynec@wsu.edu

CSIRO Librarian
Black Mountain Library
GPO Box 109
Canberra, ACT 2601, Australia

Dr. Darleen A. De Mason
Dept. of Botany & Plant Sciences
University of California
Riverside, CA 92521, USA
demason@ucr.ac.uk

Dr. Claire Domoney
Department of Metabolic Biology
John Innes Centre
Colney Lane
Norwich NR47UH, UK
claire.domoney@bbsrc.ac.uk

Dr. T.H.N. Ellis
Institute of Biological,
Environmental & Rural Sciences
Aberystwyth University
Gogerddan
Aberystwyth
Ceredigion
SY23 3EB
noe2@aber.ac.uk

Dr. Sergei Gostimskii
D-mitziya Ul'yanova St. 12/2
S.Q. 24
Moscow, 117292, Russia

Dr. Niall Green
Herbage & Vegetable Section
Scottish Agricultural Sci. Agency
82 Craigs Road
Edinburgh EH12 8NJ, UK
niall.green@sasa.gov.uk

Dr. Michael A. Grusak
USDA/ARS, CNRC
1100 Bates Street
Houston, TX 77030, USA
mgrusak@bcm.tmc.edu

Dr. Lee A. Hadwiger
Department of Plant Pathology
P.O.Box 646430
Washington State University
Pullman, WA 99164-6430, USA
chitosan@wsu.edu

Dr Julie Hofer
Institute of Biological,
Environmental & Rural Sciences
Aberystwyth University
Gogerddan Campus
Aberystwyth
Ceredigion SY23 3EB, UK
Tel: +44 (0)1970 823186
jmh18@aber.ac.uk

Debra Inglis
WSU-NWREC
16650 State Route 536
Mount Vernon, WA 98273, USA
dainglis@wsu.edu

INRA
UMR APBV
Domaine de La Motte - BP35327
35653 Le Rheu CEDEX
France

Izumi Campus Library
Tohoku Gakuin, 2-1-1 Tenjinsawa
IZUMI-Ku
Sendai 981-3193 MZ
Japan

Dr. Hans-Joerg Jacobsen
Institute of Plant Genetics
Leibniz University of Hannover
Herrenhauserstr. 2
D-30419 Hannover, Germany
Jacobsen@lgm.uni-hannover.de

John Innes Operations Center
Library & Information Service
Colney Lane
Norwich NR4 7UH, UK

Mrs. Slavka Kalupchieva
The Maritsa Veg. Crops Res. Inst.
ul Brezovsko strosse No. 32, PK20
4003 Plovdiv, Bulgaria

Dr. Oleg E. Kosterin
Institute of Cytology and Genetics
Lavrentiev Ave. 10
Novosibirsk 630090, Russia
kosterin@bionet.nsc.ru

Tony Leonforte
Agriculture Victoria Horsham
Private Bag 260
Natimuk Rd., Horsham
Victoria 3401, Australia
tony.leonforte@nre.vic.gov.au

Dick S. Lowe
Pureline Seeds Inc.
Box 8866
Moscow, ID 83843-1366, USA

Dr. Kevin McPhee
NDSU Dept. 7670
370G Loftsgard Hall
P.O. Box 6050
Fargo, ND 58108-6050 USA
kevin.mcphee@ndsu.edu

Dr. Vojislav Mihailovic
Inst. of Field and Vegetable Crops
Forage Crops Department
Makisima Gorkog 30
21000 Novi Sad
Serbia and Montenegro

Aleksandar Mikic
Inst. of Field and Vegetable Crops
Forage Crops Department
Makisima Gorkog 30
21000 Novi Sad
Serbia and Montenegro
aleksandar.mikich@gmail.com

Mision Biologica de Galicia
CSIC Biblioteca
Apartado de Correos 28
36080 Pontevedra, Spain

Montana State University
Libraries
P.O. Box 173320
Bozeman, MT 59717-3320 USA

Dr. Rachel Moreno
Life Sciences Division
Rock Valley College
3301 N. Mulford Rd.
Rockford, IL 61114
Tel: (815) 921-3487
r.moreno@ednet.rockvalleycollege.edu

Dr. Fred J. Muehlbauer
USDA, Dept. Agronomy
Washington State Univ.
Pullman, WA 99164, USA
muehlbau@wsu.edu

Prof. I.C. Murfet
School of Plant Science
University of Tasmania
GPO Box 252-55
Hobart, Tas. 7001, Australia
ian.murfet@utas.edu.au

Dr. Jim Myers
Department of Horticulture
Oregon State University
Corvallis, OR 97331-7304, USA
myersja@bcc.orst.edu

Dr. Constance G. Nozzolillo
306 Crestview Rd.
Ottawa K1H 5G6, Canada
cnozzoli@rogers.com

Dr. Florence Picard
VILMORIN
Service Documentation
49250 La Menitre, France
florence.picard@vilmoria.com

Dr. Neil O. Polans
Department of Biological Sciences
Northern Illinois University
DeKalb, IL 60115, USA
npolans@niu.edu

Polish Academy of Sciences
Mrs. B. Sadowska, Head of Library
Institute of Plant Genetics
ul. Strzeszynska 34
60-479 Poznan, Poland

Dr. Lyndon Porter
USDA-ARS, VFCRU
Veg. & Forage Crops Res. Unit
24106 N Bunn Road
Prosser, WA 99350, USA
Tel: (509) 786-9237
lporter@pars.ars.usda.gov

Dr. Serge M. Rozov
Institute of Cytology & Genetics
Russian Academy of Sciences
Lavrentjev Ave. 10
Novosibirsk 630090, Russia
rozov@bionet.nsc.ru

Serasem
Centre Agronomique
Z.A. du Port
77940 La Brosse Montceaux,
France

Dr. B. Sharma
16/10 East Patel Nagar
Delhi 110008, India

Dr. K. K. Sidorova
Institute of Cytology & Genetics
Russian Academy of Sciences
Lavrentjev Ave. 10
Novosibirsk 630090, Russia
sidorova@bionet.nsc.ru

Dr. Susan R. Singer
Department of Biology
Carleton College
Northfield, MN 55057, USA
ssinger@carleton.edu

Dr. Olga G. Smirnova
Institute of Cytology & Genetics
Russian Academy of Sciences
Lavrentjev Ave. 10
Novosibirsk 630090, Russia

Dr. Ingrid Sterner de Renzone
Salta 545, 2107
Alvarex (SF), Argentina
msterner@agatha.unr.edu.ar

Dr. Peter Smykle
SWETS Information Service
160 Ninth Avenue, Unit B
Rennemedede, NJ 08078
USA

Dr. Gail Timmerman-Vaughan
Crop and Food Research/CRI
Private Bag 4704
Christchurch, New Zealand
timmermang@crop.cri.nz

University of Tasmania
Library - Serials Section
GPO Box 252-25
Hobart, Tas. 7001, Australia

John Wamatu
W. Brotherton Seed Co
P.O. Box 1136
Moses Lake, WA 98837, USA
john@brothertonseed.com

Dr. Trevor Wang
Department of Metabolic Biology
John Innes Centre
Colney Lane
Norwich NR4 7UH, UK
trevor.wang@bbsrc.ac.uk

Dr. Tom Warkentin
Crop Development Centre
University of Saskatchewan
51 Campus Dr.
Saskatoon, SK S7N 5A8, Canada
warkentin@sask.usask.ca

Dr. Norman Weeden
Plant Science & Plant Path. Dept.
Montana State University
Bozeman, MT 59717, USA
nweeden@montana.edu

Dr. Jim L. Weller
School of Plant Sciences
University of Tasmania
GPO Box 252-55
Hobart, Tasmania 7001
Australia
jim.weller@utas.edu.au

Dr. Neil Williams
Seneca Foods Corporation
P.O. Box 100
Dayton, WA 99328, USA
newilliams@senecafoods.com
Tel: (509) 629-3106