PROCEEDINGS OF THE FOURTH INTERNATIONAL OAT CONFERENCE

VOLUME IV

MISCELLANEOUS PAPERS

Edited by: Sue Pelham and Andrew Barr

October 19th – 23rd, 1992

Hilton International Hotel
and Festival Theatre Complex

Adelaide, South Australia

Hosted and organised by the Fourth International Oat Conference Inc.
on behalf of the International Oat Conference Committee.

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Sponsored by

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PREFACE

The Fourth International Oat Conference was held in Adelaide from 19th – 23rd October, 1992.

In the business meeting at the close of the Third International Oat Conference held at Lund, Sweden in 1988, the International Organising Committee under the chairmanship of Dr Robert Forsberg was elected to organise the next conference. Adelaide was chosen as the venue. The final date of the Fourth Conference and the outline of the program, including selection of the main speakers, were suggested by the International Committee but local arrangements and finalisation of the program were delegated to a local sub-committee comprising staff from the South Australian Department of Agriculture, the Australian Barley Board, the Uncle Tobys Company and the Australian Oat Breeders Group. I wish to record my appreciation of the work done by this local committee in assuming various organisational responsibilities over the three years prior to the conference.

Special thanks to:

Sue Pelham, Tom Hoppo, and Dean Wardle: for managing the oat breeding program during conference organising.

Geof Palmer: for efficiently managing finances as Treasurer

Dick Medd: for help, advice and encouragement in all aspects of the Wild Oats in World Agriculture Symposium.

Elisabeth Eaton: Principal of the Conference Secretariat (Festival City Conventions) for outstanding service and attention to detail.

Alan Dube: arranging sponsorship for delegates from Bulgaria, India, Czechoslovakia and Poland.

David Sparrow: liaison with the Crawford Agricultural Trust and Southern Foods to assist delegates from Bulgaria and Poland.

The Conference was organised into two special symposia ("The Changing Role of Oats in Human and Animal Nutrition", "Wild Oats in World Agriculture"), two days of general sessions (Crop Protection, Molecular Biology, Breeding Methodology, Economics and Marketing, Cytogenetics/Genetics, Physiology, Forage) and a field tour of the Lower North district of South Australia.

The Fourth International Oat Conference acknowledges the generous assistance from the following sponsors:

South Australian Department of Agriculture

Crawford Agricultural Trust

Australian Tourist Commission Convention Assistance Scheme

Qantas

Australian International Development Assistance Bureau

Ansett Australia

Australian Barley Board (Oat Growers Pool)

The Uncle Tobys Company

Grains Research and Development Corporation

In addition, **The Quaker Oats Company** is a sustaining member of the IOC

PREFACE continued

A total of 178 delegates from 22 countries attended the Conference. These were made up of 110 full time, 68 one-day or two-day registrants, students and associates.

This Volume IV of Proceedings contains papers which were received after the print deadline. Abstracts of some of these papers were printed in Volumes I, II and III. After finalisation of the conference accounts, a small cash surplus was fortunately available for the printing and distribution of this volume.

I would, once again, like to thank our generous sponsors and wish the next International Oat Conference every success in advancing the knowledge, production and quality of the oat crop.

Andrew R Barr
Chairman
Fourth International Oat Conference, Inc.

Miscellaneous Papers

Table of Contents

Papers *Page*

Oat Grain Quality Effects on Oat Milling Efficiency
Gary L. Yarrow, R. Gary Fulcher and A. Bruce Roskens 1
(Volume I pp. 32)

The Use of Molecular Genetics in the Quest for Wild Oat Control
Brian Arnst 6
(Volume II pp. 35)

Gene Effects for Harvest Index in Avena sativa L. x Avena sterilis L. Oat Crosses
J.S. Verma and S.N. Mishra 8
(Volume III pp. 145)

Posters

Heavy Metal Uptake by Oat Varieties in Relation to Biomass Production in Contaminated Environment
Frantisek Macháň 12
(Volume III pp. 172)

Additional contributions

Gene Effects for Fodder Yield in Oats
S.N. Mishra and J.S. Verma 22

The Varietal Identification of Single Seed Oat by Acid Page of Avenin
Desimir Knezevic and Dragoljub Makisimovic 27

Polymorphisms of Avenin Detected by Acid Page in Wild Oat (A. fatua L.)
Dragoljub Makisimovic and Desimir Knezevic 31

Oat Grain Quality Affects on Oat Milling Efficiency

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Summary

Oat grain quality can be defined in many ways, such as the amount of contamination, percentage of a particular component, bushel weight, etc. One of the economic characteristics of grain quality is milling yield; the amount of green oats needed to make 100 kgs of oat groats or flakes. This paper summarizes the initial results of a long term study to examine the morphological characteristics of oats with known commercial milling yields. We measured test weight, groat percent, 1000 kernel weight, length (Dmax), width (Dmin), Fshape (Dmin/Dmax), volume and density of 100 kernels of 48 commercial oat mill samples. We then regressed these parameters against the measured mill yield of these samples. Test weight, groat percentage, 1000 kernel weight and density had significant regressions, however only groat percentage and density had high enough R^2s (0.56 for both) to be of use. Caution is suggested in the interpretation of these results, as more data is needed to complete this study.

Introduction

Oat quality is an all encompassing term that is used for many differing characteristics. The grower considers a "high quality oat variety", as an oat that is relatively disease resistant, low lodging, and high yielding. A nutritionist may view a high quality oat as being one that is high in a particular nutrient, vitamin or fibre component. An oat milling company examines these traits, but when describing a "high quality milling oat", will refer to an oat that has a high "milling yield". Milling yield is defined as the amount of oats needed to make 100 kgs of oat groats, and can be also written as a proportion (100 kgs/number of kgs needed to make 100 kgs of groats). In some cases, mill yield can also be defined as the amount of green oats needed to make 100 kgs of oat flakes. Milling yield is an economic factor for any milling operation and is a direct cost of producing oat products.

Through the years, oat mill yields have changed significantly. Reported values for milling yield have ranged from 240 (42%)[(2)] to 133 (75%)[(4)]. Milling yield depends upon many variables including: amount of foreign material, grain characteristics, milling equipment and expertise of the milling personnel. To predict milling yield, parameters such as test weight, groat percentage, and kernel weight have been used. Presently, most oats in the US are rated on the basis of test weight (kgs/hl).

The trait best suited as a basis to purchase milling oats has been in question for many years. As early as 1907[(3)], oat researchers have studied various grain traits looking for indicators of milling yield. The most common traits examined have been: test weight, groat percentage, and 1000 kernel weight. In a few cases, results of analysis examining kernel morphology have been reported[(1,5,6)], with various conclusions. The objective of this paper is to give initial results of a long term study to examine various easily measured parameters for use in predicting mill yield. It is our long term goal to give breeders and other oat geneticists, quantitative information that they can use as an evaluation trait in developing new varieties.

Methods

All samples used in this study were collected by milling plant personnel at The Quaker Oats Company's oat milling operation at Cedar Rapids, IA, USA. Samples were collected at approximately the same time each day when oat milling yields were being calculated. For the purpose of this paper, all mill yields are expressed as the proportion 100 kg/(quantity to produce 100 kgs of groats). These samples were then measured for test weight (kg/hl), and subsamples were collected using a Sortex Precision Grain Divider. All samples were from commercial run oats, i.e., an unknown mixture of commercially grown varieties, except for a few single day test runs of predetermined quality oats.

1000 kernel weights (KW) were determined and oats were dehulled by hand to provide an accurate groat percentage measurement. Intact kernels were then measured using a digital image analyzer[7]. Approximately 100 kernels were measured for length (Dmax), width (Dmin), and surface area. From this information, Fshape (Dmin/Dmax), density, and volume (using the formula for the volume of 2 cones, $2(1/3*Pi*(1/2Dmin*)^2*(Dmax))$, were calculated. All statistical analyses were performed using the CoStat Statistical System (CoHort Software; Berkeley, California, USA).

Results and Discussion

Table 1 summarizes data collected from 48 different samples over a period of 3 months. Since this study is ongoing, additional samples will be analyzed as they become available.

Table 1. Summary statistics for 48 oat samples.

	Mill Yield	*Area (mm^2)*	*Dmax (mm)*	*Dmin (mm)*	*Fshape*
Average	0.68	21.02	9.64	2.96	0.31
Minimum	0.55	19.73	9.19	2.85	0.30
Maximum	0.77	22.43	10.18	3.08	0.34
StDev	0.05	0.73	0.20	0.7	0.01

	Test Weight (kg/hl)	*Percent Groats*	*1000 Kernel Weight*	*Volume (mm^3)*	*Density (gms/cc)*
Average	54.39	75.58	30.62	44.2	0.69
Minimum	45.74	68.79	23.21	40.1	0.55
Maximum	59.47	79.00	34.76	49.2	0.77
StDev	2.84	2.99	2.50	2.4	0.06

After the data was collected on the above 48 samples, linear regressions were calculated between mill yield and each measured parameter, and are shown in Table 2.

To determine if the variability of individual kernels within a sample influenced mill yield, regressions were also calculated between mill yield and the standard deviation of each parameter. No significant regressions were found (data not shown). Although four parameters had significant R^2s, only groat percentage and density had a high enough value to be used as an oat mill yield estimator.

Although the regressions for test weight, groat percent, 1000 kernel weight and density were highly significant, plots (figures 1-4 overleaf) of the data indicate that two distinct groups of samples are present. One group consisted of six samples having distinctly lower mill yield than the remaining samples. We feel that it is necessary to examine additional samples before any general conclusions from this data can be made.

Table 2. Regression values for measured traits vs. milling yield.

Parameter	R^2	*Significance level*
Area	0.06	NS
Dmax	0.07	NS
Dmin	0.03	NS
Fshape	0.001	NS
Test weight	0.28	<0.1%
Groat percent	0.56	<0.1%
1000 kernel weight	0.36	<0.1%
Volume	0.06	NS
Density	0.56	<0.1%

If the data from the low mill yield samples are removed, the groat percent and 1000 kernel weight regressions become non-significant, while Dmin, Fshape and test weight are significant (Table 3). Although statistically significant, all R^2s for Dmin, Fshape, test weight, and volume, are too low to be of any use to an estimator of mill yield.

Table 3. Regression analysis of data set with low mill yield samples removed.

Parameter	R^2	*Significance level*
Surface Area	0.08	NS
Dmax	0.01	NS
Dmin	0.18	0.5%
Fshape	0.16	0.9%
Test weight	0.18	1.6%
Groat percent	0.01	NS
1000 kernel weight	0.06	NS
Volume	0.11	3.0%
Density	0.01	NS

The quality of cereal grains is determined by any number of characteristics. For a company like the Quaker Oats Company, these traits are field production, processing and manufacturing, functional, organoleptic and physiological/biochemical. Various groups within Quaker study these parameters from a number of different perspectives. Our primary objective of the current study, is to provide guidelines for oat geneticists in producing new varieties.

Quaker's involvement in oat research began in the late 1930's. The primary purpose was to provide assurance that an adequate supply of superior quality oats would be available for purchase. This effort was initiated, in part, because the level of public funding of oat research was inadequate for rapid advancements. The primary objectives of much of this research was high yielding and disease resistant varieties. However, relatively little direction has been given to oat geneticists as to what oat traits are important for processing companies such as Quaker Oats.

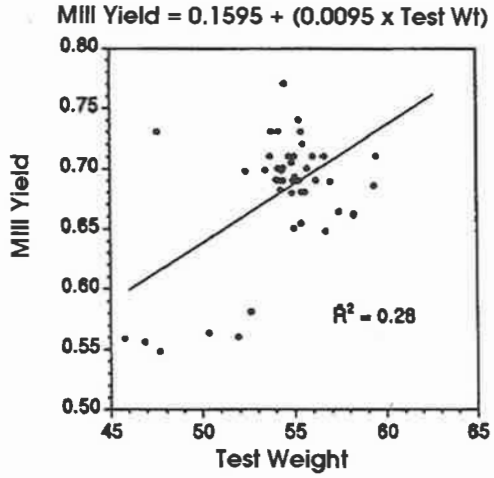

Figure 1. Mill Yield vs Test Weight (KG/HL)

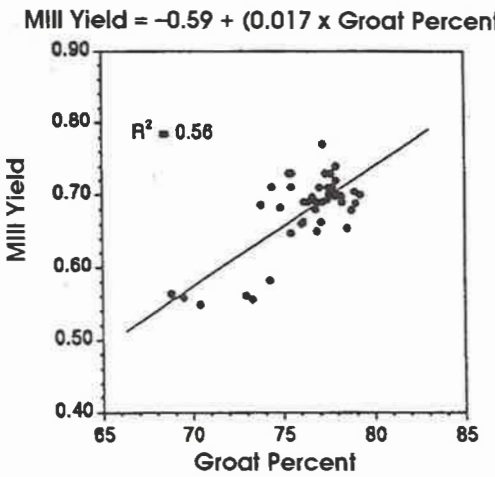

Figure 2. Mill Yield vs Groat Percent

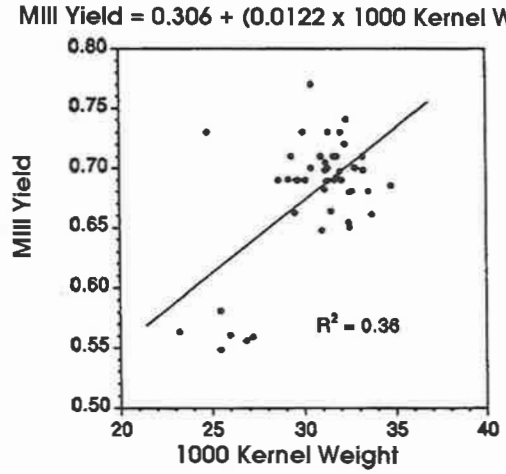

Figure 3. Mill Yield vs 1000 Kernel Weight (Gms)

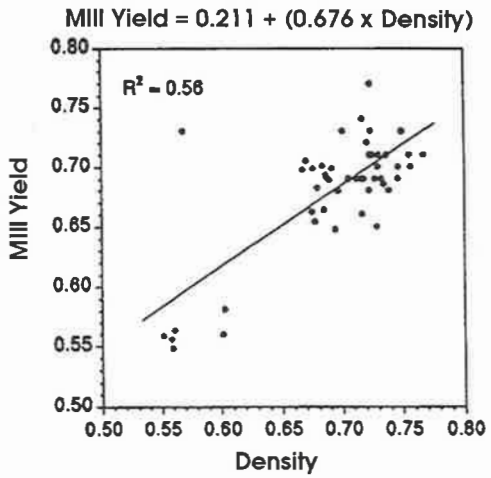

Figure 4. Mill Yield vs Density (Gms/mm^3)

In the late 1960's and early 70's, the perceived need for higher protein cereals in the human diet evolved into a focus for oats research. During this time, Quaker Oats grain buyers paid a premium for high protein, which lead to an emphasis for oat breeders to produce high protein oat varieties. This emphasis was very successful, however due to regulatory issues and consumer demands, claims for high protein products are no longer a high priority issue. U.S. oat research again focused on grain yield and disease resistance.

New nutritional information during the 1980's, in regards to fibre, brought a new emphasis to oats. Many federal, state and private programs across the country are now concerned with the soluble fibre content of oats, especially β-glucan.

Stemming from the 1980's "oat miracle food" claims, many new mills were built and old ones converted and updated. However, a severe drought in 1988 brought about a panic as domestic oat sources disappeared and new foreign sources became very valuable. A myriad of oat quality from new regions of the US, Canada, South America and Europe were obtained and evaluated. It was during this evaluation that the U.S. market began dealing with a new, very high quality oat from Finland and Sweden that gave unprecedented U.S. mill yields. From this experience, oat buyers began to take active interest in a different type of "value-added" oat, that is, one with increases in milling yields. Savings in a few kilograms of mill yield may be the deciding factor in profitability for a milling operation.

The present study examined many common morphological traits to determine what traits are important in determining mill yield. From the results, it appears that size characteristics have little influence, and the classical tests have a very strong influence in the oat milling process. Over 56% of oat milling yield can be explained by groat percentage.

This is not surprising since the oat hull was determined to comprise 21 to 32% of the oat grain in this study. Others have reported ranges of 25 to 35%. Oat hull percentage is influenced by genotype, position on the panicle, and genotype by environment interactions[4,9]. A theoretical maximum yield can be estimated by groat percentage alone, however this does not take into account other variables such as thin oats, bosom oats, light oats or the particular genotype's seed size variability. All of these oat types increase the variability of oat coming into the mill, which makes "fine tuning' the mill processes difficult. We believe that one reason the Finnish and Swedish oats have such high mill yields, is the high degree of kernel size uniformity.

At this time, we do not believe we have enough information to make any strong conclusions on what parameters can best be used as a milling yield indicator. It is hoped that continuation of this study will give a better, quantitative parameter. Our current plans are to continue this investigation using the present techniques. We will include analysis of both domestic and foreign grown oats. In addition measurements on pure varieties (including mill yield evaluation) will be done. Discussions with oat millers indicate that the ease of dehulling is a vital component of mill yield, thus we plan to design experiments to test if this can be quantitatively determined. It is also planned to develop a method of examining a more thorough 3-D analysis, and examine its relationship to milling yield.

Acknowledgements

The authors thank Kate Harrigan, U of Minnesota, for the digital image analysis work, and Joseph Lucas, Richard Wilson, and Larry Stewart of the Quaker Oats Company, for collection of samples.

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The Use of Molecular Genetics in the Quest for Wild Oat Control

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Wild oats are one of the worst weeds in major cereal growing regions of the world. Their effect on cereal yields and methods of control are well documented in the literature and are discussed elsewhere in this symposium. In several countries, and particularly in Australia, there is an increasing incidence in the number and magnitude of reports of resistance of grass species to the commonly used post emergent grass herbicides. Resistance of wild oats to diclofop-methyl was recorded in Western Australia in 1985[2]. As diclofop-methyl is the most widely used herbicide for wild oat control this raises concerns about future control strategies. While cross resistance to other selective grass herbicides has not been fully investigated the potential exists based on previous experience with annual ryegrass, *Lolium rigidum*[3].

There is no doubt that herbicides will continue to play a major role in wild oat control, despite the presence of resistance, through development of management systems involving the use of fallows, knockdown herbicides and herbicide mixtures. Recent developments in the new technology field of genetic engineering however suggest that it may be possible in the future to use herbicide tolerant crops as a means to treat potential resistant weed species with non-specific herbicides.

In 1971, Monsanto released the chemistry of a new class of herbicide, glyphosate as Roundup® herbicide, which has since become the most widely used pesticide in the world. This nonselective herbicide has a unique mode of action which may explain why, despite its widespread use, there has been no recorded instance of resistance occurring.

The primary mode of action of glyphosate is inhibiting the shikimic acid pathway and stopping the production of aromatic amino acids, essential building blocks for plant growth. Glyphosate actually competes directly with 5-endopyruvylshikimic acid-3-phosphate (EPSP) synthase, an enzyme of the shikimic acid pathway[1], resulting in starvation of the plant and ultimately its death. This mode of action is key to the understanding of the development of glyphosate tolerance through genetic engineering.

Since 1982, Monsanto has taken a leading role in the development of biotechnology in the area of plant sciences, especially for agricultural crops. Since that time more than fifty plant species have been genetically engineered[4]. The method most commonly used relies on the ability of a plant pathogenic bacterium, *Agrobacterium tumefaciens*, to transfer parts of its DNA into plant cells. It does this by introducing genes into parts of its DNA fragments which integrate into the chromosomes. This induces the cells to produce elevated levels of hormones, ultimately forming galls on the plant. Monsanto scientists were able to remove the disease causing genes from the bacterium and replace them with genes for other specific traits. Bacteria containing the desirable trait are then placed in solutions along with leaf discs from the selected crop plant. The bacteria infect the cells of the leaves which are in turn grown into complete plants using the techniques of tissue culture.

This method works well for many dicots which are natural hosts for the *Agrobacterium tumefaciens* bacterium. However, it is not successful for grain crops such as rice, maize and wheat. Using these techniques Monsanto, has been able to introduce insect tolerance, virus tolerance and herbicide tolerance into several key agricultural crops.

The first step in developing Roundup tolerance occurred in 1983. Researchers from both Monsanto and Calgene isolated genes for ESPS synthase from bacteria and plants. Developments from this work produced gene constructs which produced higher amounts of these proteins in plants. As previously mentioned, glyphosate, (Roundup), competes with ESPS synthase preventing amino acid production. In transformed plants, where higher levels of ESPS synthase are produced, the amount of glyphosate in the plant is unable to compete at the sites in the shikimate pathway, thus enabling the plant to tolerate the herbicide.

While initial work was carried out on tomatoes, Monsanto have now progressed several crops including canola, soybeans, cotton and potatoes towards commercialisation.

As mentioned earlier, transformation of monocots has proven much more difficult than dicots. Many methods of injecting DNA into both protoplasts and intact plant cells have been tried unsuccessfully. It was not until 1987, when workers at Cornell University developed a DNA particle gun, that successful transformation of monocots was achieved. This device uses minute tungsten particles which are coated with DNA to bombard plant cells. Through multiple bombardment it is possible to get some of the desirable DNA into the plant cell.

In 1988, rice transformation was achieved followed in 1990 by maize. It was only recently in 1992 that wheat transformation has been accomplished in a collaborative effort between Monsanto and the University of Florida. The first transgenic wheat plant was produced by particle gun delivery of DNA to embryonic wheat callus[6]. pBARGUS DNA was bombarded into a highly embryonic callus tissue and selected for its ability to grow on a Basta (glufosinate) impregnated medium. Progeny from this plant was obtained by embryo rescue after outcrossing to a wild type wheat plant. Transformation was demonstrated in the R1 progeny by Southern analysis, PAT enzyme assays and resistance to Basta.

The production of a transgenic wheat plant is a major milestone. However, wheat transformation still remains very difficult[5]. Much of the difficulty lies in the *in vitro* tissue culture and regeneration systems as well as the genes and selectable markers available for wheat. Many of the culture systems that have made the transformation of other cereals possible are not yet available in wheat.

At Monsanto we are working on improvements in the tissue culture, delivery of DNA to cells and on gene expression to wheat. We have a suspension system to produce stably transformed wheat callus. The transformation of wheat will become a routine procedure only after significant improvements are made in both tissue culture and the expression of genes in wheat. Monsanto has developed the genes for high resistance to glyphosate in monocots and along with the developments in transformation this trait will be introduced into wheat in the near future.

Being able to develop the technology is the first major hurdle to be overcome. However, this does not mean an automatic path to commercialisation. The technology is new and perhaps frightening to the majority of the public who are uninformed of the low risk and high benefits it will bring. In many countries there are strong public movements pressuring governments to slow down and even stop the commercialisation of such technology. Aligned with this is the need for some regulatory framework to be developed to at least assure the public that any evaluation and subsequent commercialisation is done in a truly scientific and well planned manner.

More specifically, if Roundup tolerant wheat is commercialised, how do we control the volunteer cereals that are seen each year in the pasture and fallows following cropping? In todays farming systems the majority of these are controlled with Roundup. The decision to proceed will need to be carefully made.

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Gene Effects for Harvest Index in *Avena Sativa* L. x *A. Sterilis* L. Oat Crosses

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Summary

Three *Avena sativa* L. cultivars (Kent, Nodaway and WA 1470), and one *A. sterilis* L. accession (PI 295932) were used for developing the experimental material. The gene effects were estimated using the parental (P1 and P2), F1, F2, and backcross (B1 and B2) generation means of each of the three interspecific crosses, viz., Kent x PI 295932. Estimates of different genetic parameters[2] indicated additive, dominance, additive x additive and dominance x dominance effects were highly significant in the cross Kent x PI 295932. In the other two crosses viz., Nodaway x PI 295932 and WA 1470 x PI 295932, the additive effect was more predominant than the dominance effect. However, both estimates were highly significant. Early generation selection and intermating among F2/F3 generation selected plants is proposed for further improvement in the harvest index.

Introduction

The ratio of grain yield to biomass is harvest index. The use of *Avena sterilis* introductions from the Middle-East in oat breeding programmes has been common in recent years. This material tends to be a good source of genes for high biomass and high vegetative growth index[4]. Improvement in grain yield of cultivated oats from introductions of *A. sterilis* germplasm is due primarily to increased vegetative growth index. Vegetative growth index and harvest index accounted for more than 90% of the variation in grain yield among oat lines from *A. sativa* x *A. sterilis* crosses[11]. Harvest index is an efficient criterion for indirect selection of grain yield in oats[10]. Since the ratio of grain yield to biomass equals harvest index, so the product of biomass and harvest index equals grain yield. Biomass yield can be increased by producing a higher vegetative growth index. Therefore, knowledge about the genetic control of biomass yield and harvest index should aid breeders to genetically manipulate these traits in developing higher yielding cultivars.

Materials and Methods

Three *Avena sativa* cultivars; namely Kent, Nodaway and WA 1470, were each crossed with one *A. sterilis* accession viz., PI 295932 which was used as the male parent. The populations developed from each of the three crosses i.e. Kent x PI 295932 (cross I), Nodaway x PI 295932 (cross II) and WA 1470 x PI 295932 (cross III), were parental (P1 = *A. sativa* parent and P2 = *A. sterilis* parent), F1, F2, and backcross (B1 = F1 x P1 and B2 = F1 x P2) generations. Two rows of parental, 1 row of F1, 7 rows of F2 and 3 rows each of B1 and B2 generations were planted in a compact family block design. Depending upon the expected variances of generations, 5 to 45 plants were selected randomly from each replication to record observations on biomass yield i.e., dry plant weight (g) of a bundle of culms cut at ground level at maturity and grain yield (g) per plant for the ultimate calculation of harvest index.

The joint scaling test[2] was applied to test the genetic models as well as for estimating the parameters of the models. Additive-dominance and digenic-interaction models were fitted successively for all the characters. If the goodness of fit yielded a significant chi-square value i.e., non-fit for the additive-dominance model, the possibility of a digenic-interaction model would then be employed.

Results and Discussion

Generation means of the different crosses for biomass yield, grain yield and harvest index are presented in Table 1. F1 means were higher than those of both the parents for biomass yield and grain yield in all three crosses. For harvest index, F1 means were higher than mid-parental values in cross II and III and higher than both the parents in cross I. A comparison of F1 means with parental means indicated the presence of dominance and/or epistasis for these characters. Overdominance was evident from higher values of F1 over the corresponding better parent for all traits except for harvest index in cross III. Higher values of F2 means than the mid-parental values indicated the existence of residual heterosis.

Table 1. Generation means for biomass, grain yield and harvest index in three interspecific oat crosses.

Generation	*Biomass yield (g)* Cross			*Grain yield (g)* Cross			*Harvest index (%)* Cross		
	I	*II*	*III*	*I*	*II*	*III*	*I*	*II*	*III*
P1	112.3	67.7	70.6	36.8	21.4	24.8	31.8	31.5	35.1
P2	75.0	71.8	75.0	18.0	71.1	17.8	24.2	23.9	23.8
F1	116.7	98.4	101.6	37.7	28.8	32.5	32.3	29.9	32.1
F2	107.3	89.4	96.2	32.1	26.1	28.1	29.6	29.7	28.8
B1	114.4	84.0	99.9	36.5	27.7	31.1	31.9	32.9	31.1
B2	112.7	96.1	102.4	25.1	21.2	23.4	23.2	22.1	22.8
S.Em.+	6.40	5.14	5.83	2.23	1.85	2.21	1.15	2.23	0.98
CD(0.05)	23.19	18.62	21.12	11.70	6.70	8.00	3.64	7.05	3.07

The χ test of goodness of fit for additive-dominance (3-parameter) and digenic-interaction (5-parameter, i.e. excluding the parameter "j") models were fitted to the generation means for biomass yield, grain yield and harvest index. The χ values for the additive-dominance model were significant ($P<0.001$) for biomass yield in cross I and III and for harvest index in cross I. Non-significant ($P>0.01$) values were observed for the digenic-interaction model for biomass yield in cross I and III and for harvest index in cross I, indicating the adequacy of the digenic-interaction model.

The estimates of gene effects under the adequate model are presented in Table 2. The main effects, additive ("d") and dominance ("h") were found to be highly significant for harvest index and grain yield in all the three crosses and for biomass yield in cross II only. However, in crosses I and III only additive effects were found to be significant for biomass yield. Additive effects were more pronounced than dominance effects for harvest index in crosses II and III. The results showed a preponderance of additive gene effects for harvest index and biomass yield. An inheritance study involving *Avena sativa* x *A. sterilis* crosses indicated that harvest index seemed to be controlled primarily by additive gene action[6]. Similar results, from the studies involving *sativa* and *sterilis* oat matings, were reported by various other workers[4,10,11]. The large amount of additive effects indicates that substantial progress can be made using standard selection in the development of pure line varieties. Dominance effects were higher in magnitude than additive effects for grain yield in all the three crosses and for harvest index and biomass yield in cross I and II respectively. Inheritance studies[10] also observed considerable dominance effects for grain yield and biomass yield in oats. In all three crosses the positive sign of parameter "h" indicated dominance for high harvest index.

The results for grain yield per plant showed the significance of only additive and dominance gene effects in all three crosses. However, the dominance effect was of higher magnitude in all cases. The positive value of the dominance effects revealed that high grain yield was dominant. Preponderance of dominance effects has been reported earlier[8,10].

From a combining ability study of *A. sativa* x *A. sterilis* oat matings it was observed that gene action for grain yield was largely additive[3]. Later reports indicated that introgression of "yield genes" from *A. sterilis* into cultivated oats (*A. sativa*) caused an ultimate improvement in grain yield[4].

Table 2. Estimates of gene effects under adequate model in three interspecific oat crosses.

Parameter	*Biomass yield (g)* Cross			*Grain yield (g)* Cross			*Harvest index (%)* Cross		
	I	II	III	I	II	III	I	II	III
m	115.4** ±8.4	70.0** ±0.5	107.7** ±7.2	27.4** ±0.4	19.3** ±0.3	21.2** ±0.3	20.5** ±2.4	27.6** ±0.5	28.2** ±0.6
(d)	18.6** ±0.6	2.2** ±0.5	2.1** ±0.8	9.4** ±0.4	2.2** ±0.3	3.5** ±0.3	4.3** ±0.4	4.2** ±0.5	6.5** ±0.6
(h)	1.3 ±8.6	30.0** ±1.6	-6.0 ±7.3	10.2** ±0.6	9.2** ±0.7	11.4** ±0.8	11.7** ±2.5	2.2** ±0.5	2.9** ±1.0
'i'	-21.7* ±8.5	-	-34.7** ±7.3	-	-	-	7.8** ±2.5	-	-
'l'	-38.5** ±10.4	-	3.9 ±10.5	-	-	-	8.4** ±3.0	-	-

* P = 0.05, ** P = 0.01

Among the digenic interactions (epistatic effects), additive x additive ("i") and dominance x dominance ("l") contributed to the inheritance of harvest index and biomass yield in cross I but the magnitude of the latter was higher, while only additive x additive was significant for biomass yield in cross III. Most probably there is no report on oats showing the occurrence of epistatic gene effects for harvest index. The obvious discrepancy in the findings may be due to the difference in the materials and the methodology used. In a 6 x 6 diallel study in oats, overdominance and gene interaction was observed for plant dry weight, i.e., biomass yield[1]. From a combining ability study of interspecific oat matings it was noted that gene action for biomass yield was largely additive[3]. Analysis of data on biomass yield in oats indicated that biomass was controlled largely by additive gene action[5].

Considerable improvement might be expected if selection is practiced in early segregating generations for harvest index in crosses II and III. The chances for such improvement in cross I are, however, not as favorable because of the existence of non-fixable effects. Considering these results, fixable as well as non-fixable effects may be utilized through selection in the early segregating generations, exploiting the fixable effects first. Simultaneously, breeding methods like modified recurrent selection[9] and intermating among F2/F3 generation selected plants[7,3] may be tried for the exploitation of such gene effects. The proposed system of early generation selection and intermating will ensure full utilization of additive, dominance and epistatic gene effects and will lead ultimately to fixation of a given character at the desirable level.

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Heavy Metal Uptake by Oat Varieties in Relation to Biomass Production in Contaminated Environment

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Summary

A high level of contamination of the environment, particularly soils and their saturation with heavy metals can be found even at locations with a lower industrial load. Sludge from wastewater treatment plants is an important source of heterogeneous substances. With high nutrient rates, oats with its powerful root system is able to produce high amounts of biomass and to transform heavy metals into forage as well as into grain. This transformation is different in individual varieties. Average increases in dry matter were 62% as compared with a control. The highest uptake of heavy metals by oat plants was determined at the growth stage DC 31. Lower cadmium (Cd) uptake was determined in Zlaťák and Auron varieties at the period of green-matter harvest, lower copper (Cu) uptake in Ardo and Zlaťák, and the lowest zinc (Zn) uptake in the Zlaťák variety. A correlation analysis revealed significant relationships between biomass production and heavy metal uptake at particular growth stages. Relatively lower values of Cd uptake by a grain were determined in the Flämingsnova variety; responses to Cu and Zn uptake and accumulation in a grain were different.

Introduction

Varietal differences in the response of oats to contaminated environments can be related to their different morphological structures. Under favourable growing conditions, especially water content in the soil, there are large increases in oat biomass[23]. The highest biomass production was determined in oat varieties at the heading and maturing stages[16,18,19,27].

Over the last years, a higher saturation of environment (particularly the soil) with heterogeneous substances has appeared. Depending on the kind of substance and agroecological conditions, they are accumulated in higher amounts in the soil and enter into a plant system through the root system or leaf area[1,20].

The most important sources of soil contamination are sludge from wastewater treatment plants, industrially produced compost, fertilizers, etc.[4,25].

Inputs of heterogeneous substances into the processes of food production are decreased by prevention (decreasing environmental contamination) and using species and varietal tolerance to absorb these substances into the organisms.

For decreasing the load of cadmium in soils (which is one of the most dangerous heavy metals), the following methods are used: chemical[4,17], chemical and biological using a detoxication agent on the basis of a humic acid fraction into the soil (Kolář, pers. comm., 1991), and biological, based on genetic relationships of heavy metals uptake by a plant[28], and particularly on tolerance to the uptake by plant species[6,9,12,22,31]. Wojcieska[32], Beneš[3], Dunemann *et al*[7], Bláha and Sixta[5], Wettlaufer *et al*[29] investigated Cd uptake by oat plants.

Critical values of heterogeneous substance contents in soils and plants are given in decrees by the Czechoslovak Ministry of Agriculture and Federal Committee for Environment, especially for feed-stuffs, including feeding cereal crops. Approximate values for fodder grain are as follows: Cd 0.3 mg kg^{-1}, Cu 50, Zn 250, Mn 100, Cr 3, Pb 2, Hg 0.1 mg kg^{-1}. Contents of heavy metals in sludge from a wastewater treatment plant significantly surpass critical values. The sludge is reported to contain dioxin, polychlorinated biphenyls and total amount of organic chloride compounds[2].

Some of the heavy metals can be considered essential if they occur in low concentrations. However, they are toxic when contained in higher concentrations. Lead, mercury and cadmium are not the elements essential for human life, they are toxic and besides that they are accumulated in the environment.

Cadmium is one of the most important and dangerous heavy metals. It affects functional and morphological changes in organisms. Its toxic effects are explained by inhibition of enzymatic systems. A natural Cd content in soils ranges from 0.01 to 15 ppm. A toxic content for plants is 10 ppm. The sludge has a higher Cd content depending on sources. Under conditions of Cu deficiency, photosynthetic intensity and plant activity are decreased depending on the length of Cu differentiation, age and development stage of plants. With a high supply of Cu, a large amount of assimilates enter into oat panicles and kernels, while with a low Cu supply assimilates remain in vegetative organs, particularly in leaf blades. Cu uptake by a plant is inhibited by a higher chromium (Cr) concentration in the environment. Zinc, besides copper, iron and manganese, is one of less important heavy metals that play a basic role in plant life. Concentrations of Zn reported by Tiller[26] are from 1 to 100 mg kg^{-1} of biomass. High concentrations are already toxic. Translocation of Zn into plants is straight[14]. A deficiency of Zn in roots affects transport and distribution of phosphorus (P) into a stem and reduces total plant dry matter[31]. Wilke[30] studied the role of Zn in the soil up to the amount of 500 mg kg^{-1} for dehydrogenase activity. High rates of soluble Zn inhibited its activity significantly. According to Singh[22] and Gezsin and Bayrakli[8] the influence of Zn on plants and their yields are in relation to its concentration in tissues, soil pH and organic carbon, in particular under arid and semi-arid conditions.

This paper is aimed at evaluating Cd, Cu and Zn uptakes by oat varieties and their effects on green matter and grain formation.

Materials and Methods

Oat varieties Zlaťák ((Tarpan x Fläm.krone) x Pan), Flämingsnova (Pendek x Fläm.stahl), Ardo ((Fläm.nova x Pan) x Thor x Saturn)) and Auron ((Fläm.nova x Pan) x Veles) are grown on 95% of the oat sowing area in CSFR. Their agronomic traits are given in Table 1.

Table 1. Agronomic traits of oat varieties - ÚKZÚZ 1989-91

Variety	Grain yield*	Lodging**		Plant height	Growing season - days	
		I	II	(cm)	Heading	Maturity
Fl.-nova	101	7	4	116	83	135
Zlaťák	98	7	6	119	84	137
Ardo	102	7	5	126	85	138
Auron	106	7	6	116	84	138

* 100% = 5.82 t ha^{-1} ** 9 = no lodging

The response to heavy metal uptake by plants of these varieties was evaluated in pots, under natural environmental conditions. Ten plants were cultivated in a pot; each variant was in three replicates. The experiment was conducted through two growing seasons. The contaminated substrate consisted of municipal sludge from a wastewater treatment plant and silicic sand in a 2:1 ratio. Control variants consisted of the soil from the fields of the Cereal Research Institute Kroměříž and mixed in the same ratio. The analysis of sludge was made using AAS_1 and is presented in Table 2.

Table 2. Chemical composition of soils for testing heavy metal uptake by oat varieties.

Element	*Control*	*Municipal sludge VN*$_{1-2}$
Dry matter, %	83.4	28.17
Total N, mg kg^{-1}	1637	17376
P, mg kg^{-1}	82	764
K, mg kg^{-1}	162	464
pH/KCl	5.7	7.1
Cadmium, mg kg^{-1}	0.43	20.00
Nickel, mg kg^{-1}	0.10	50.00
Zinc, mg kg^{-1}	200.00	1500.00
Lead, mg kg^{-1}	27.70	30.00
Chromium, mg kg^{-1}	11.60	255.00
Copper, mg kg^{-1}	60.00	550.00

The Agrochemical Enterprise Kroměříž[21] monitored an annual pollution with cadmium (4 g ha^{-1}) and lead (24 g ha^{-1}) through rainfalls at a location studied. The other heavy metals from the air (Ni, Cr, Cu) were not studied.

Dry matter production and the uptake of Cd, Cu and Zn which significantly surpassed a standard were determined at 4 growth stages according to Zadoks *et al*[33], i.e. DC 31, 49, 81 and 91. Mean samples were analyzed for heavy metal contents in plant dry matter and grain[10].

Results and Discussion

Saturation of the environment with heterogeneous substances is irregular and depends on an exposed location, distances away from sources and weather conditions. Even under conditions with lower industrial load, such as the Kroměříž district, an annual Cd pollution through rainfall is variable and ranges from 20 to 80 g ha^{-1} (Fig. 1). The importance of local sources of heavy metals for environmental contamination is given in Table 3. Contents of heavy metals were higher - Cd 10 times, Zn and Cu 5 times and Cr twice as high as compared with critical values. The sludge contained a high proportion of total nitrogen substances and higher proportions of P and potassium (K) in comparison with the control soil. The results correspond with findings by Beneš[3] who considers sludge from wastewater treatment plants as the biggest sources of soil pollution with heavy metals.

Oat plants positively responded to a luxury supply with nutrients through their powerful root systems. They increased biomass production and dry matter even at the initial growth stages. An average increase in biomass production of the 4 investigated varieties was 50 % in green matter and 60 % in dry matter as compared with control variants. The highest total differences in increases in green and dry matter were determined in Zlaťák and Ardo varieties (Fig. 2). The Auron variety was sensitive; it produced smaller increases in both green and dry matter. This is confirmed by comparing relative growth rate[13] in varieties and variants (Fig. 3, 4).

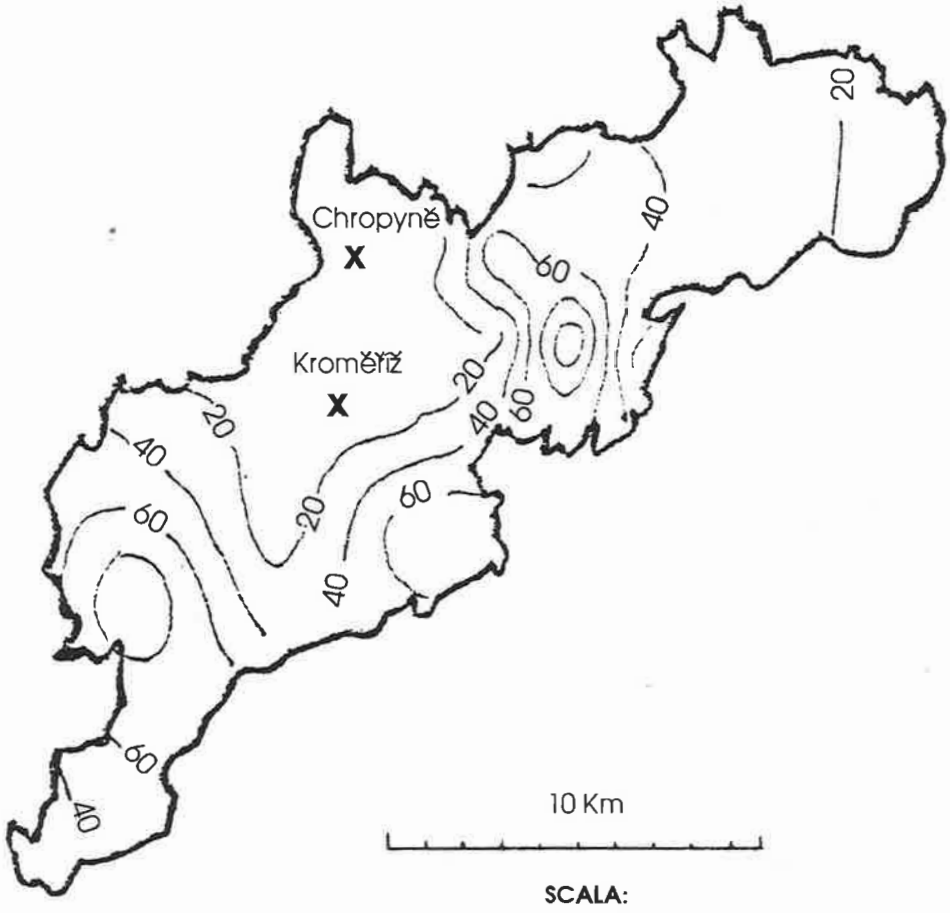

Figure 1. Annual pollution of cadmium through rainfall (g ha^{-1}).

Table 3. An analysis of municipal sludge VN_{1-2} for heavy metal content and comparison with critical values (1992).

Element	*Content* *mg kg^{-1}*	*Critical values* *I mg kg^{-1}*	*II mg kg^{-1}*
Cadmium - Cd	20	2	4
Nickel - Ni	50	50	70
Zinc - Zn	1500	300	600
Lead - Pb	30	100	300
Chromium - Cr	255	100	300
Copper - Cu	550	100	400

A correlation analysis of interactions between fresh biomass and dry matter of all varieties confirmed the relationship with Cd uptake (-0.59* and -0.54*), and in dry matter with Cu uptake (-0.74**) as well. All varieties showed high sensitivity to heavy metal uptakes (Cd, Cu, Zn) at the growth stage DC 31. There were varietal differences in the course of the uptake at subsequent stages.

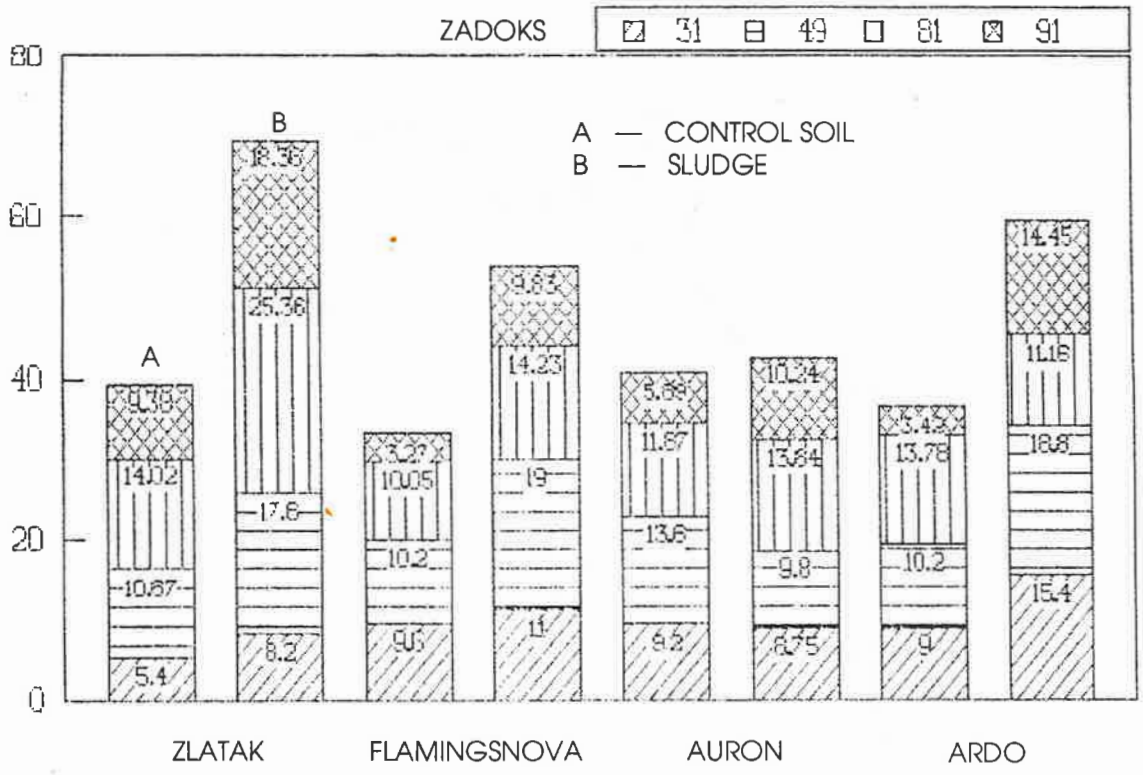

Figure 2. Dynamics of oat green matter production (g plant^{-1}).

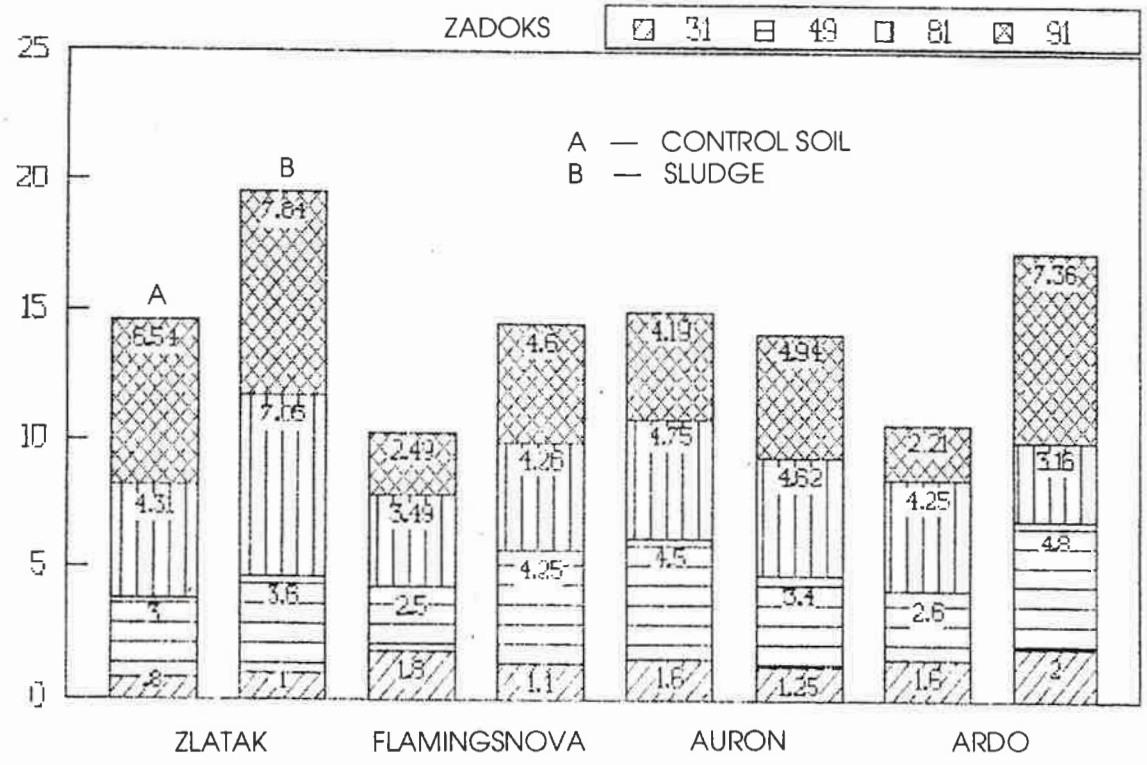

Figure 3. Dynamics of oat dry matter production (g plant^{-1}).

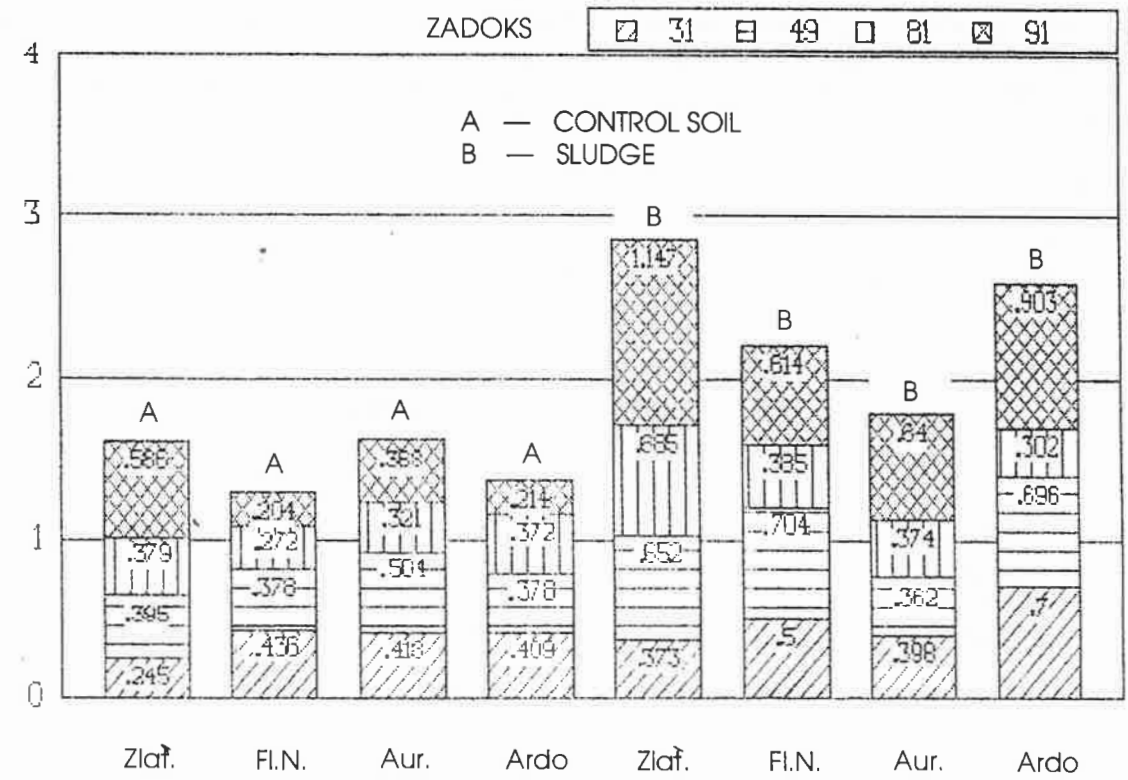

Figure 4. Increase in oat biomass production (g plant^{-1} day^{-1})

Cadmium

Analyzed plant samples at the growth stage DC 31, which had Cd supply *ad libidum*, showed an increase from 0.29 to 0.56 mg kg^{-1}. The lowest Cd uptake was determined in the Ardo variety. Correlation relationships were found between Cd and Cu contents (r=0.91*), and Cd and Zn contents (r=0.78*).

There was a disproportion between the increase in biomass and Cd uptake at the subsequent growth stage (DC 49). In all varieties the level of absorbed Cd in dry matter reduced in the range of 0.07 (Zlaťák) to 0.18 mg kg^{-1} (Ardo); the average increase in dry matter was 30%. This fact is confirmed by a determined high correlation between dry matter through green matter production and dry matter (r=0.86**), while there was no significant correlation with Cd uptake. Intervarietal differentiation in Cd uptake by oat plants occurred at the stage DC 81. Zlaťák and Auron varieties showed a decreasing trend of uptake. When the increase in dry matter of the Zlaťák variety was 64%, the difference in Cd uptake as compared with a control was +0.17 mg kg^{-1}. The increase in dry matter of Auron reduced by 3% when compared with a control, and the difference in Cd uptake decreased to 0.03 mg kg^{-1}. The increase in dry matter was 14% on average of all varieties. The highest correlation was found between Cd content and number of tillers (r=0.85**).

At the stage of full ripeness, the increase in dry matter in sludge substrate was still 46% in comparison with DC 81. The highest increase was found in the Ardo variety (4.20 g) due to its positive response to a higher supply with nutrients. Intervarietal differences in Cd uptake continued to increase, however the Zlaťák and Auron varieties kept low values.

Correlation analyses in varieties did not show any significant relationships in Cd uptake into astem due to high intervarietal differences. Thus Zlaťák and Auron maybe considered more suitable varieties for the green matter harvest[15] at locations with higher Cd contamination.

The Cd content in a grain of a variety was remarkably balanced (Table 4). A lower Cd uptake by the Flämingsnova variety is associated with a lower plant weight. It is confirmed with a highly significant correlation between Cd uptake into a grain and productive tillering ($r=0.97^{**}$), and a correlation between biomass weight and Cd uptake (0.85^{**}). The correlation analysis revealed that Cd uptake into a grain was influenced by Zn content in a stem ($r=0.96^{**}$).

The results show a high Cd uptake by oat grain. Only the Flämingsnova variety with its Cd uptake being 0.37 mg kg^{-1} is close to a newly prepared standard for feeding cereal crops (0.3 mg kg^{-1}). The other varieties surpassed this standard nearly twice when they had Cd uptake *ad libidum*. These results correspond with findings reported by Beneš[3].

Copper

At the growth stage DC 31, a high Cu content in sludge was absorbed equally by the oat varieties. The Auron variety showed a higher uptake (9.31 mg kg^{-1}) as compared with a control variety.

At the stage DC 49, there was a differentiation in the uptake among varieties. A slight increase was found in the Ardo variety (7.13 mg kg^{-1}), the other varieties showed a falling trend in uptake. The higher the biomass increase was, the lower the Cu uptake was by plants. The biggest decrease in uptake was determined in the Auron variety (0.29 mg kg^{-1}).

Cu uptake continued to decrease at the stage DC 81 and it reached lower values in Zlaťák and Ardo than in a control. Correlation analyses did not show relationships between any of the traits studied.

For locations with a higher Cu contamination, Ardo and Zlaťák varieties may be recommended for green-matter harvest.

At the ripening stage (DC 91), the Ardo variety showed the highest decline of Cu uptake (-3.82 mg kg^{-1}) which was lower in sludge than in a control variant. Intermediate values in the uptake were detected in the Auron variety (2.56 mg kg^{-1}). Higher Cu uptakes were determined in Zlaťák and Flämingsnova varieties, nevertheless they were acceptable.

A phenotypic correlation showed that Cu content in a stem significantly influenced its transport into a grain ($r=0.90^{**}$). Owing to a low Cu content in a grain, Ardo is the most suitable variety. The other varieties studied did not surpass the standard for Cu content either, which is 50 mg kg^{-1}.

The investigations revealed that Cu uptake by oat plants was not high and, subject to longer testing under field conditions, oats may be grown at locations with an increased Cu content in the environment.

Zinc

Zn content in sludge significantly surpassed critical values. At the stem elongation stage (DC 31), Zn uptake was remarkably different in individual varieties. These differences in varieties, when compared with control variants ranged from 130 to 480 mg kg^{-1}. The highest uptake was found in Flämingsnova and Zlaťák varieties as well as in their control variants.

Decreasing Zn uptake was characteristic for the whole growing season until the milk-ripeness stage. Their values compared with controls were below 70 mg kg^{-1} in Ardo, Zlaťák and Auron varieties. The varieties mentioned, in particular the Zlaťák variety, could be more suitable for locations with a high Zn content in the environment.

Zn translocation into a grain depended on the variety and was different for each variety. The lowest uptake was assessed in the Zlaťák variety and ranged from 50 to 60 mg kg^{-1}. Zn uptake in the other varieties surpassed a standard twice.

Conclusion

Oats is able to absorb an efficient amount of nutrients by its root system and transform them into biomass on the condition of adequate rainfall. Among cereals, oats reaches the highest increases in biomass production, however as a spring crop it has a limited period of production in relation to biomass quality[15,24] for feeding purposes.

Corrected values of increasing absorption of heavy metals by oat varieties from environment production are given in Table 4. Macháň *et al*[16] recommended Zlaťák and Ardo varieties for green matter feeding. Both of these varieties gave higher yields of green matter, however, they had different Cd, Cu and Zn uptakes. The Auron variety had a lower Cd uptake and at the same time reduced biomass production.

Table 4. Corrected values of increasing the heavy metal uptake by oat biomass from environment as compared with control, Kroměříž, 1991.

Variety	*Biomass production* ($t\ ha^{-1}$)	*Cd* content ($mg\ kg^{-1}$)	*Cd* uptake ($g\ ha^{-1}$)	*Cu* content ($mg\ kg^{-1}$)	*Cu* uptake ($g\ ha^{-1}$)	*Zn* content ($mg\ kg^{-1}$)	*Zn* uptake ($g\ ha^{-1}$)
Zlaťák	20.43	0.12	2.45	1.52	31.05	61.95	1265.64
Fl.-nova	17.61	0.16	2.82	3.73	65.69	97.49	1716.80
Auron	16.35	0.07	1.14	1.78	29.10	77.27	1263.36
Ardo	20.50	0.26	5.33	3.09	63.35	68.18	1397.69

Correcting the increase in values of heavy metal uptake by a grain (Table 5) is important for their penetration into processes of food production. Grain yields correspond with varietal productivity and results reported by Palik *et al* (1991). Differences in heavy metal uptake into a grain are illustrated in Figure 5. Relative increases in the values of Cd uptake by a grain were balanced in all varieties except for Flämingsnova. There were varietal differences in Cu and Zn uptakes.

Table 5. Corrected values of increasing the heavy metal uptake from environment by oat grains in relation to controls, Kroměříž, 1991.

Variety	*Grain yield* ($t\ ha^{-1}$)	*Cd* ($mg\ kg^{-1}$)	*Cd* ($g\ ha^{-1}$)	*Cu* ($mg\ kg^{-1}$)	*Cu* ($g\ ha^{-1}$)	*Zn* ($mg\ kg^{-1}$)	*Zn* ($g\ ha^{-1}$)
Zlaťák	3.39	0.48	1.63	5.27	17.86	16.05	55.93
Fl.-nova	3.36	0.34	1.14	4.19	14.08	79.94	268.60
Auron	3.60	0.48	1.73	2.91	10.48	117.87	424.33
Ardo	3.49	0.48	1.67	3.50	12.22	93.75	327.18

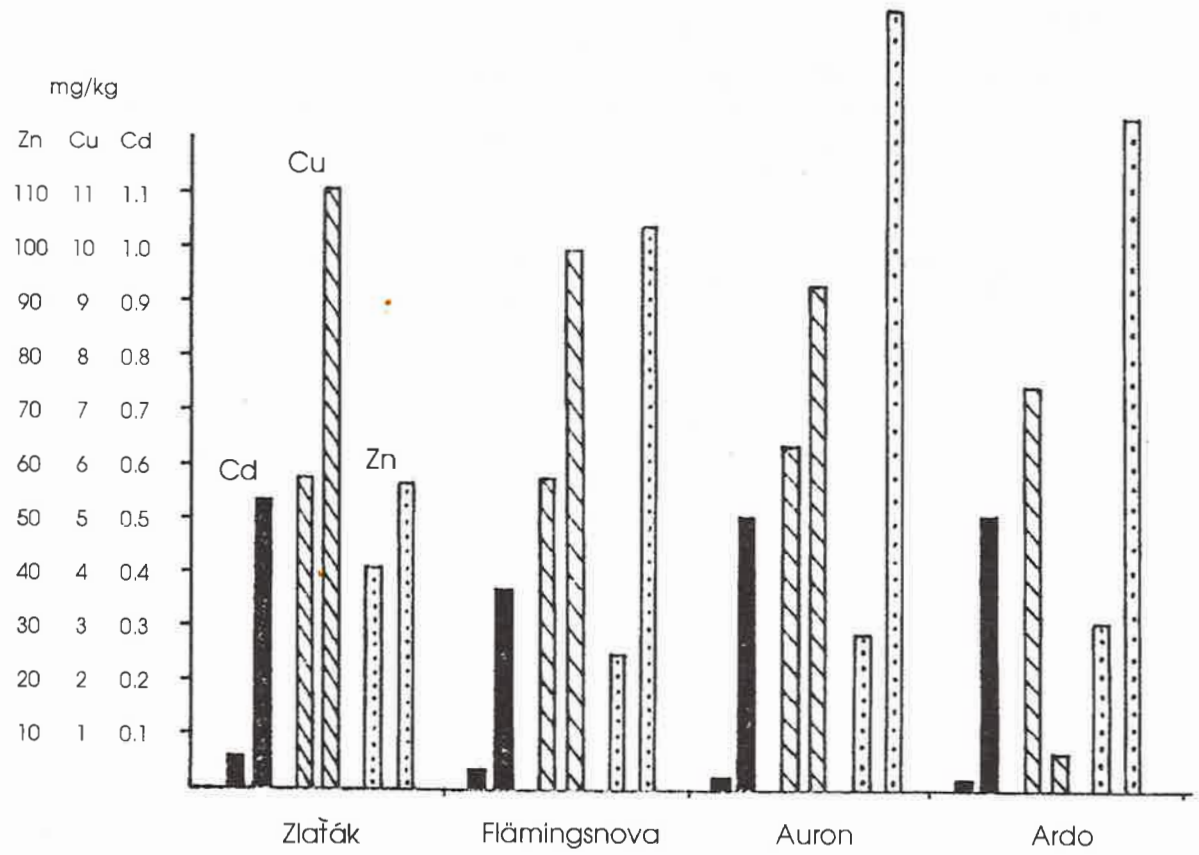

Figure 5. Heavy metal content in oat grains, Kroměříž.

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Gene Effects for Fodder Yield in Oats

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Summary

Parental, F2, F3, B11, B22, B1s and B2s generations were developed for each of the three inter-specific crosses of *Avena sativa* L. cultivars Kent (K), Nodaway (N), and WA 1470 (W) with an *A. sterilis* accession PI 295932 (P). Gene effects were estimated using generation mean analysis for green fodder and dry matter yields. Additive and dominance effects were significant for both the traits in all the three crosses except in KxP, where only the dominance effect was significant for green fodder yield. Among the epistatic effects, additive by additive and dominance by dominance were significant for green fodder and dry matter yields in KxP. The epistasis observed was of duplicate type.

Introduction

Hybridization of cultivated with wild relatives of plants has resulted in the improvement of crop species[3]. Introgression of genes from weedy and wild relatives into cultivated oats (*Avena sativa* L.) breeding programmes has been common in the recent years. *Avena sterilis* L., the wild progenitor of cultivated oats[2], tends to be a good source of genes that confer resistance to crown rust. It has also been identified to have genes for high yield, greater total leaf area and later onset of senescence, along with high biomass and high vegetative growth index[4,7,9]. These traits, coupled with high tillering, quick regeneration capacity[1,6] and resistance to trampling, form a strong base for breeding forage oat varieties suited to multiple cuttings/grazing. To date, no assessment has been made on gene effects utilizing generation mean analysis in interspecific crosses of oats. Therefore, a study was made to determine gene effects from a set of three *A. sativa* x *A. sterilis* crosses in the ongoing oat breeding programme utilizing generation mean analysis.

Materials and Methods

The experimental populations were derived from crosses of three *A. sativa* cultivars, Kent (K), Nodaway (N) and WA 1470 (W) as females with an *A. sterilis* accession, PI 295932 (P) as male. The parents involved in the three interspecific crosses were previously evaluated for their important agronomic attributes in various experiments in the ongoing oat breeding programme over a period of three years and were found to be widely divergent. Important characteristics of these parents are given in Table 1. The parental, F2, F3, B11, B22, B1s and B2s generations were produced and grown in a randomized complete block design. The experiment consisted of three replications with each replication composed of 35 experimental rows as follows:

Population/generation	*Number of rows*
Parental	2
F2	7
F3, B1s and B2s	6 each
B11 and B22	4 each

Each row was 3 m long and spaced 25 cm apart. Within each row seeds were sown at a spacing of 10 cm. Data were recorded on green fodder and dry matter yields in kg per meter row length at 50% heading stage. The data were subjected to generation mean analysis and a joint scaling test[5] was applied to test the adequacy of genetic models and for estimating the genetic parameters, the mean (m), and five specific types of gene action: additive (d), dominance (h), and the epistatic effects: additive by additive (i), additive by dominance (j), and dominance by dominance (l).

Table 1. Important characteristics of *A. sativa* cultivars and the *A. sterilis* accession used as parents.

Parental line	*Origin*	*Plant height (cm)*	*Days to heading (50%)*	*Tillers/plant*
A. sativa				
Kent	USA	135-140	106	7
Nodaway	USA	120-125	110	8
WA 1470	Australia	100-105	100	6
A. sterilis				
PI 295932	Middle East	165-170	104	11

Results and Discussion

Mean performance

Mean performance of eight generations with respect to green fodder and dry matter yields are given in Table 2. The F2 and F3 means exceeded the parental performance for green fodder yield in crosses KxP and NxP, whereas it was not so in the cross WxP. B11 and B22 also showed superior performance in green fodder yield over parental, F2 and F3 performance in KxP. However, this trend was not seen in crosses NxP and WxP. For dry matter yield, the F2 and F3 performance were almost the same as parental, but B11 and B22 means were higher than parental, F2 and F3 means in cross KxP. This superiority was again not apparent in crosses NxP and WxP. Table 2 shows that the F2 and F3, and B11 and B22 population means of the three crosses were in the following order for both green fodder and dry matter yields:

KxP		NxP		WxP
F2	>	F2	>	F2
F3	>	F3	>	F3
B11	>	B11	>	B11
B22	>	B22	>	B22

Looking at the means, it appears that the cross KxP is the most potential one as far as extraction of superior pure breeding lines are concerned. It further suggests that more interspecific crosses with *A. sterilis* should be attempted to generate more genetic variability for the improvement of green fodder and dry matter yields as this species seems to possess genes for high herbage yield also.

Table 2. Mean performance of different generations for green fodder and dry matter yields in three *A.sativa* x *A. sterilis* crosses.

Generation[#]	Green fodder yield (kg) KxP	NxP	WxP	Dry matter yield (kg) KxP	NxP	WxP
P1	2.64 ±0.23	1.87 ±0.10	1.20 ±0.05	0.52 ±0.01	0.26 ±0.01	0.27 ±0.01
P2	2.49 ±0.19	2..28 ±0.01	2.23 ±0.13	0.36 ±0.01	0.34 ±0.01	0.36 ±0.00
F2	2.62 ±0.24	2.34 ±0.33	1.41 ±0.15	0.45 ±0.00	0.33 ±0.03	0.26 ±0.02
F3	2.71 ±0.10	2.38 ±0.32	1.34 ±0.08	0.41 ±0.00	0.34 ±0.03	0.24 ±0.01
B11	3.10 ±0.10	1.92 ±0.20	1.33 ±0.14	0.61 ±0.06	0.25 ±0.02	0.23 ±0.01
B22	2.97 ±0.13	2.16 ±0.01	1.55 ±0.16	0.49 ±0.00	0.28 ±0.01	0.28 ±0.00
B1s	2.52 ±0.16	2.45 ±0.21	1.30 ±0.18	0.41 ±0.02	0.38 ±0.02	0.23 ±0.01
B2s	2.56 ±0.22	2.26 ±0.12	1.46 ±0.22	0.44 ±0.03	0.31 ±0.02	0.26 ±0.01

[#] P1 = *A. sativa* cultivars, P2 = PI 295932 (*A. sterilis*), F2 = F1 of P1 x P2 selfed, F3 = F2 selfed, B11 = B1 x P1, B22 = B2 x P2, B1s = B1 selfed, and B2s = B2 selfed.

Gene effects

Estimates of gene effects for green fodder and dry matter yields are presented in Table 3. Model fitting results showed that for the generations derived from the cross KxP, only the 6-parameter model was adequate to explain variation in green fodder yield. The additive (d) component is almost negligible in this cross which may probably be due to high differentiation between Kent and PI 295932. Among the epistatic components, (i) and (l) were significant with (l) having a negative sign suggesting a duplicate type of interaction. This cross also exhibited a positive and high dominance component (h), suggesting dominance of increasing effect alleles. In the other two crosses, NxP and WxP, the 3-parameter model (m,(d), and (h)) was adequate. In both these crosses the sign of the dominance component was negative and almost twice that of (d), indicating dominance of decreasing effect alleles. Among these two crosses the additive component was higher in WxP (0.41) than NxP (0.14).

For dry matter yield, the 6-parameter model (m,(d),(h),(i),(j) and (l)) was adequate in crosses KxP and NxP, whereas only the 3-parameter model was adequate in the cross WxP. In all three crosses, the additive components were lower than their respective dominance components. The dominance component was positive (0.62) in the cross KxP whereas it was negative in crosses NxP (-0.51) and WxP (-0.23). This suggests dominance of increasing effect alleles in KxP and dominance of decreasing effect alleles in WxP.

As additive by additive and dominance by dominance components have opposite signs in crosses KxP and NxP, the duplicate type of epistasis is operating for dry matter yield in these two crosses.

In a breeding programme involving *A. sativa* and *A. sterilis* genotypes and the type of gene actions estimated, it is quite apparent that the cross KxP has the most potential as compared to NxP and WxP in the improvement of green fodder and dry matter yields. It is suggested that to utilize such crosses more successfully it may be desirable to follow modified recurrent selection, i.e. alternating pedigree and recurrent cycles of selection for the exploitation of such gene effects. Presence of the duplicate type of epistasis in the crosses may, however, hinder the progress of selection and hence adoption of biparental matings in F2/F3 generations or a diallel selective mating system with or without modification may prove to be useful in isolating high performing lines from these crosses.

Table 3. Estimates of gene effects with standard errors under adequate model for green fodder and dry matter yields in three *A. sativa* x *A. sterilis* crosses.

Cross	*Gene effects*						*Chi-square*
	m	*(d)*	*(h)*	*(i)*	*(j)*	*(l)*	
				Green fodder yield			
KxP	1.90 ±0.27	0.07 ±0.15	4.32** ±1.38	0.65** ±0.23	-0.07 ±0.73	-5.81* ±2.59	5.21 (NS)
NxP	2.12 ±0.04	0.14** ±0.04	-0.26** ±0.11				9.34 (NS)
WxP	1.63 ±0.06	0.41** ±0.06	-0.73** ±0.27				8.48 (NS)
				Dry matter yield			
KxP	0.27 ±0.06	0.08** ±0.01	0.62** ±0.23	0.16** ±0.05	-0.33** ±0.10	-0.55** ±0.27	4.80 (NS)
NxP	0.46 ±0.06	0.04** ±0.01	-0.51* ±0.21	-0.16** ±0.05	-0.16* ±0.08	0.48 ±0.33	5.28 (NS)
WxP	0.31 ±0.00	0.04** ±0.00	-0.23** ±0.01				9.56 (NS)

* and ** = significant at the 5 and 1 percent probability levels respectively.

The limitations of the present study have been two fold. Firstly, the F1s were not included in the study and therefore the magnitude and sign of the dominance component and also the number of parameters adequate to explain the variation for a trait may change on the basis of the expectation of the F1. The coefficient of the dominance component is 1.0 (F1 = m+(h)). Secondly, in this study, because the parents of two different species are involved, reciprocal effects could explain some of the variation which could not be ascertained due to lack of reciprocal crosses and generations derived from them. Significant interactions of *A. sterilis* cytoplasm with *A. sativa* matings have indicated that yield increase may be due to specific favourable interactions between *A. sativa* nuclear genomes and cytoplasm[8]. Also, it is necessary to include more accessions of *A. sterilis* to identify better sources of genes for improving forage productivity of cultivated oats on the basis of gene effects, combining ability and selection of parents for interspecific matings throwing transgressive segregates.

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The Varietal Identification of Single Seed Oat by Acid Page of Avenin

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Summary

The avenin fraction of oat was separated into 46 components by polyacrylamide gel electrophoresis in sodium lactate buffer at pH 3.1. Different cultivars possess different combinations of these protein components. Differences between cultivars were found according to the number of components and their relative mobilities. Electrophoretic formula of avenin for analysed oat cultivars are presented to help in the identification of cultivars from the electrophoregrams.

Introduction

It is well known that various methods of electrophoresis can be used to analyse the storage proteins and discriminate between cultivars of a cereal species. Electrophoretic methods appear to be suitable methods for identifying cultivars of cereals (wheat, barley) by analysis of the grain proteins[1,2,5,8,9]. Oat cultivars have received much less attention, although there is the possibility of using the seed proteins of oats for cultivar characterisation, in a manner analogous to wheat and barley.

Oats generally have lower amounts of prolamin (avenin) and larger amounts of globulin type proteins than wheat or barley grains. Avenin of oat have been shown to demonstrate polymorphism.

Thus there is considerable scope for using electrophoretic methods for oat cultivar identification. Cooke and Cliff[2] have demonstrated how a modified version of the lactate PAGE system used for barley cultivar identification, can be used to differentiate between oat cultivars. Avenin extracted from single de-husked oat grains in 25% chloroethanol, can be separated into 13 polypeptide components by lactate PAGE using 12% acrylamide gels. Cultivars have different combinations of these components and 35 UK cultivars have been classified.

In this paper, we report the use of PAGE in aluminum lactate buffer at pH 3.1 to separate avenin proteins. The avenin banding patterns of 12 cultivars are presented. A key is developed to assist in the identification of oat cultivars, based on the presence of avenin components, their relative mobilities and color intensities.

Materials and methods

Samples of oat (*Avena sativa L.*) seed were obtained from the *Avena* collection of the Institute for Small Grains in Kragujevac, Yugoslavia. Single oat grains were manually de-husked, crushed and transferred to a polypropylene centrifuge tube. The avenin proteins were extracted by adding 200 µl 45% ethanol, vigorously mixing and then allowing to stand at room temperature for at least 2 hours. Following this, contents of the tubes were centrifuged at 4000 x g for 15 minutes. The clear supernatants were separated in new tubes and equal amounts of 40% (w/v) sucrose in aluminum lactate buffer, pH 3.1 were added. The prepared samples were used for electrophoresis.

Aliquots of 15 µl of avenin proteins were loaded into each place and electrophoresis carried out on an 8.5% polyacrylamide gel using aluminum lactate buffer, pH 3.1, at 460 V for about 1 hour. Staining was carried out with 0.05% Comassie Brilliant Blue R-250 in 12% TCA solution. After staining, the gels were photographed. Photographs were used for measuring migration distance of electrophoretic bands from the origin of the migration.

Relative mobilities (Rm) of electrophoretic bands were calculated using cultivar Rajac as a standard electrophoregram with a reference band of arbitrary relative mobility of 50. Relative mobilities were computed using the formula: $Rm = l_x \times 50 / l_{50}$, where l_x is the distance of band x from the origin of migration, and l_{50} the distance of band 50 from the origin of migration (Table 1). Relative mobilities were mean values of two different electrophoretic runs. Relative intensities of the electrophoretic bands were estimated visually, ranging from 1 to 5, with relative band intensity 1 being lightest and 5 darkest. The electrophoregram of the cultivar is thus expressed by relative mobilities and relative intensities of electrophoretic bands.

Table 1. Avenin electrophoregrams of analysed oat cultivars expressed numerically. *Relative band mobility indicated by Ri score numbers.*

	30	40	50	60	70	80	90	95
Mediteran	1 4	3 3	4	2 2	1	2	2	1 3 4 4
Slavuj	3 3	3	1 2 2	1 1 1		2	2	1 1 4
Rajac	3 4	2 4	2 5 1	1 2 1 1	2	3	2	2 4 4
Labud	3 4	3 3	2 5 2	2 2 1 1	2	3	2	2 4 3 2
Condor	3 4	3 3	2 5 2	2 2 1 1	2	3	2	2 4 3
Mustang	2 4	1 3	2 5 1	2 2 1	2	2	1	2 3 3 2
Gambo	3 3	1 3 1 2	1 1	1 1	2	2	1	3 3 1
Wels	4 2	4 2 5	1	2 2 2 1		3	1	1 4 3 1
Astor	2 4	2 3 2 5	2	2 1 1	1	2	1	1 3 2
Hermes	43	1 3 2	1 1	1 1 2	2	2	1	1 3 3
Tiger	1 4	2 3 1 4 1	5	2 1 1	2	3	2	2 4 3
Pan	4 4	2 3 2 5 2	2	2 2	3	3	1	1 3 3
	30	40	50	60	70	80	90	95

Relative band intensity 1 is lighest, 5 is darkest. All avenin bands were found between 31 and 96 units.

Results and Discussion

The results obtained using the acid PAGE procedure are shown in Figure 1. Three groups of avenin type proteins are readily discernible according to their mobilities. In the fast moving region of the gel these have been labelled **A**-avenins, medium moving avenins have been labelled **B** and slow moving proteins labelled **C**-avenins.

By comparison with earlier work[6] it is probable that the **A**-group proteins are not true avenins, but represent proteins which co-extract, and they appeared sufficiently reproducible. There are some differences between certain cultivars in this region, therefore these proteins could be considered for characterisation of oats cultivars. Oats proteins from the **A**-region can be separated into 26 possible components.

The proteins of **B** and **C** regions have been found to be most useful for cultivar differentiation, being strongly staining and consistently present. Figure 1 shows that the avenin proteins of oat cultivars can be separated into a number of polypeptides by acid PAGE; the **C** region containing 13 and the **B** region 10 possible components. Different cultivars possess different combinations of these polypeptide components and hence this could form the basis for distinguishing between cultivars.

The mobilities of the 46 avenin polypeptides have been measured and expressed as relative mobilities, using the sixth protein band found in the cultivar Rajac as a standard (Figure 1).

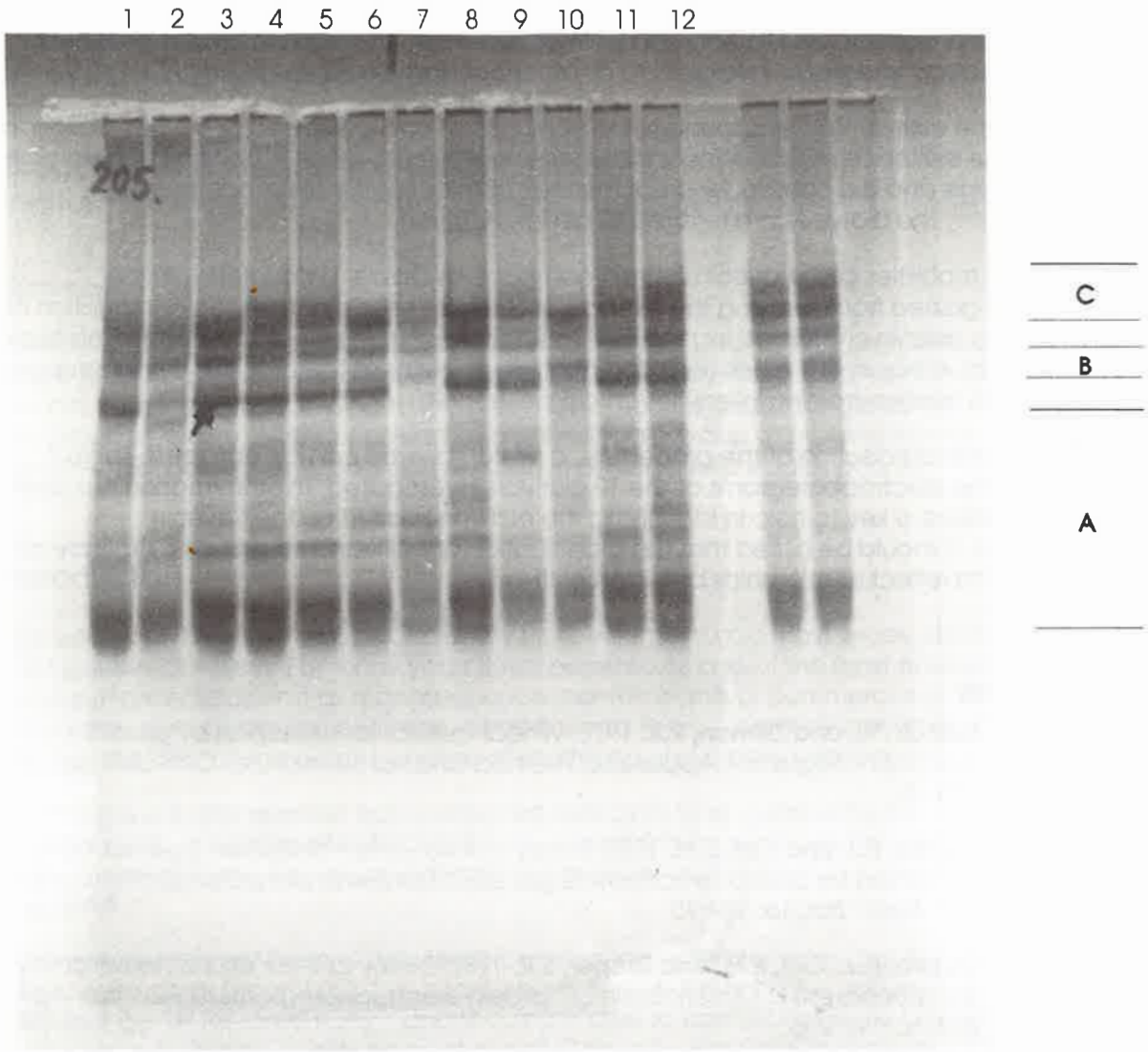

Figure 1. Electrophoregrams of analysed oat cultivars

1	Mediteran	4.	Labud	7.	Gambo	10.	Hermes
2.	Slavuj	5.	Condor	8.	Wels	11.	Tiger
3	Rajac	6.	Mustang	9.	Astor	12.	Pan

This band was chosen because it occurs in the majority of the cultivars investigated as an intensively staining component. It has been assigned an arbitrary relative mobility of 50. The relative mobilities of the other components and their relative intensities are given in Table 1.

Classification of oat cultivars

The foregoing results clearly demonstrate that the avenin proteins of oats offer a convenient and powerful method for characterising cultivars. Acid PAGE analysis of the avenin fraction indicates that there is a substantial degree of polymorphism within the avenin proteins, as has been previously reported[6,7]. This polymorphism can be exploited to provide a system for classifying oats cultivars, as cultivars differ in their avenin composition.

The analysis of 50 single seeds of each cultivar should allow the detection of variants present as 5% or more of the total at a 95% level of confidence[4]. Within these limits, the majority of cultivars examined were uniform electrophoretically. According to the avenin composition it is possible to group oat cultivars with similar electrophoregrams. In this way cv. Mediteran, Condor, Astor and Tiger belong to the same group.

Very similar electrophoregrams were shown by the pairs of cultivars cv. Rajac and Mustang, cv. Labud and Condor, cv. Gambo and Hermes, while two cultivars Slavuj and Wels had different electrophoregrams in relation to all other oat cultivars (Table 1).

However, one cultivar Pan was found to be non-uniform and possessed two types of avenin patterns. The existence of such lines or biotypes has previously been reported in wheat and barley cultivars and is a consequence of the lack of selection for electrophoretic uniformity[3,10]. The biotypes are referred to as Pan A, B, etc.

The relative mobilities of the avenin protein components (Table 1) coupled with the information gained from studying the extracts of 50 single seeds, permits the construction of a scheme to assist in characterising the cultivars. This is achieved by analysing the gels from each cultivar, measuring the positions and relative mobilities of the avenin components and assessing the relative staining intensities on a 5 point scale.

By suitable standardisation of the procedure, a useful scheme can be obtained. Table 1 represents the electrophoregrams of the 12 cultivars investigated. This information has been used to produce a key to help in identifying the cultivars, based on their avenin composition. It should be noted that the classification of cultivars into groups is arbitrary and is designed to reflect relationships between cultivars.

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Polymorphisms of Avenin Detected by Acid Page in Wild Oat (*A. fatua* L.)

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Summary

Avenins in wild oat were studied by means of acid PAGE. The avenin protein fraction of single wild oat grains (*Avena fatua*) is presented. This study showed some differences in avenin composition according to the presence of some bands in single kernel electrophoregrams. Avenin appears as a polymorphic group of proteins from a biochemical point of view and can be used in phylogenetic studies of oats.

Introduction

The wild oat (*Avena fatua* L.) is decidedly the best known and most widely distributed of wild species of *Avena*. For many years it has persisted as one of the most troublesome weeds in grain fields and as a consequence, contaminants of commercial grain. *Avena fatua* is an annual plant, a common, noxious weed in grain producing regions of the world, and is most difficult to control because of shattering and marked dormancy.

Avena fatua is distinguished from cultivated oats by its long, geniculate awns and by seed shattering via abscission of individual florets. *Avena fatua* genotypes frequently have dark coloured seed (grey, brown, or red) and pubescence on the lemma, rachilla and base of the seed.

Avena fatua has not been widely used for improving cultivated oats, but it could be used to improve some agronomically important characters. *A. fatua* and *A. sativa* may have different genes for some traits[4] and should be able to contribute genes for improving agronomic and grain quality characteristics. Generally, increases in protein yield of cereals are achieved wholly through increases in grain yield. Although this approach has increased grain and protein yields, it has failed to take advantage of genetic variability for protein content (McFerson and Frey, 1990). The avenin composition is controlled by genetic factors, not by the growing conditions.

The variability of alcohol soluble proteins of *Avena*, is studied by means of gel electrophoresis[3,5]. Moreover, avenins are found to constitute a good biochemical material with which to investigate species relationships or to identify varieties as well as interspecific variability. Because of variability and a relatively small number of avenin constituents, the oat prolamin constitutes a useful tool for phylogenetic studies of the genus *Avena*. Avenin components have molecular weights from 20,000 to 34,000 daltons and similar amino acid composition[2].

In this work the variability of the avenin composition of *Avena fatua* L. was investigated.

Materials and Methods

Samples of oat (*Avena fatua* L.) seed were collected from a grain field of the Institute for Small Grains in Kragujevac, Yugoslavia. Single oat grains were manually de-husked, crushed and transferred to a polypropylene centrifuge tube. The avenin proteins were extracted by adding 200 µl 45% ethanol, vigorously mixing and then allowing to stand at room temperature for at least 2 hours. Following this, the contents of the tubes were centrifuged at 4000 x g for 15 minutes. The clear supernatants were separated in new tubes and equal amounts of 40% (w/v) sucrose in aluminum lactate buffer, pH 3.1 were added. The prepared samples were used for electrophoresis.

Aliquots of 15 µl of avenin proteins were loaded into each place and electrophoresis carried out on an 8.5% polyacrylamide gel using aluminum lactate buffer, pH 3.1, at 460 V for about 1 hour. Staining was carried out with 0.05% Comassie Brilliant Blue R-250 in 12% TCA solution. After staining, the gels were photographed. Photographs were used for measuring migration distance of electrophoretic bands from the origin of the migration. Relative mobilities (Rm) of electrophoretic bands were calculated using cultivar Rajac as a standard electrophoregram with a reference band of arbitrary relative mobility of 50. Relative mobilities was computed using the formula: $Rm = l_x \times 50 / l_{50}$, where l_x is the distance of band x from the origin of migration, and l_{50} the distance of band 50 from the origin of migration (Table 1). Relative mobilities were mean values of two different electrophoretic runs. Relative intensities of electrophoretic bands were estimated visually, ranging from 1 to 5, with relative band intensity 1 being lightest and 5 darkest. The electrophoregram of the cultivar is thus expressed by relative mobilities and relative intensities of electrophoretic bands.

Results and Discussion

The electrophoretic pattern of Figure 1 shows the variability of avenin in mobility and intensity. Variability was not registered in the number of bands. The oat prolamins (avenins) of 200 seed samples corresponding to *Avena fatua* demonstrates 12 bands. On the gel we can recognise three different regions of avenin mobilities[1]. Figure 1 shows that the avenin of *Avena fatua* can be separated into a number of polypeptides by acid PAGE. In the fast moving region, oat prolamins (A-avenins) were separated into 6 bands while avenin in the B-region and C-region were each separated into 3 bands.

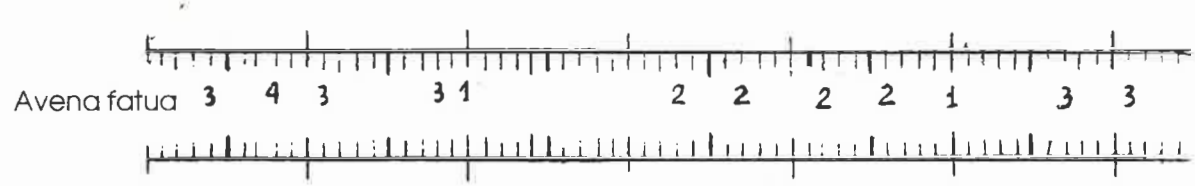
There are some differences between seed electrophoregrams on the basis of mobility and colour intensity of the fastest components. It was found that 73 oat kernels were characterised by a little faster and more intensive band than the fastest A-avenin components of the 127 remaining seeds. In this study of avenins extracted from 200 kernels of *Avena fatua* we have shown in the slow region of A-avenins at least one different band of A-avenins according to mobility.

The mobilities of 12 avenin polypeptides have been measured and expressed as relative mobilities using check cultivar Rajac as a standard which contains an intensive staining component with a relative mobility of 50 (Table 1).

The variability within *Avena fatua* in electrophoretic patterns of oat prolamins suggests polymorphism for polypeptide chains of the different bands[3]. The genetic regulation of electrophoretic constituents have been clearly demonstrated by investigation of cereal prolamins and we know the gene location which controls synthesis of wheat and barley prolamin components[6,7,8].

The above results suggest that there is a similarity between *Avena fatua* and *Avena sativa* according to their electrophoretic patterns. Knowledge of synthetic mechanisms and biochemical properties of avenin could be useful for phylogenetic studies of the genus *Avena*.

Table 1. Avenin electrophoregrams of analysed *Avena fatua* expressed numerically. Relative mobility indicated by Ri score numbers.



Relative band intensity 1 is lightest, 5 is darkest. All avenin bands were found between 32 and 91 units.

Figure 1. Electrophoregrams of analysed *Avena fatua*.

References

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