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THE *IN SILICO* PREDICTON OF THE CHLOROPLAST MATURASE K GENE POLYMORPHISM IN SEVERAL BARLEY VARIETIES

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This study was conducted to describe the role of the chloroplast maturase K (*mat*K) genetic polymorphism in the reciprocal crossing between five barley varieties using several *in vitro* / *in silico* tools. Besides, the final consequences of the *mat*K gene polymorphism on its protein structure, function, and interactions were predicted computationally. Five parental varieties were crossed to each other by full reciprocal crossing design, DNA was extracted from seeds and two different primers' pairs were designed to scan *mat*K gene. Then, polymerase chain reaction – single-stranded conformation polymorphism (PCR-SSCP) were performed. Two distinct haplotypes in both parents and artificial F1 hybrids in the *mat*K gene were observed in both amplified fragments. This finding indicated that the studied gene had no participation in the reciprocal crossing performed. Three SNPs were identified; two of them are non-synonymous (nsSNPs), namely G387V and L459M. The effect of these missense mutations on the matK protein was analyzed by several *in silico* tools. It was shown that the coding SNP, L459M was predicted to have much more effective consequences on matK protein structure and function. While the I-Mutant 2.0 prediction tool showed a decrease in stability for these two nsSNPs, which may destabilize the protein interactions to some extent. In conclusion, though the observed missense mutations in the *mat*K gene have no suggestive role in the reciprocally crossed barley varieties, they caused dramatic alterations in several matK protein moieties, which may lead to potential subsequent changes in the matK protein-mediated RNA splicing mechanisms.

Key words: chloroplast, *in silico*, *in vitro*, *Hordeum vulgare*, maturase k

Barley is highly variable in adaptation and utilization alone has caused barley to be subjected to more physiological and genetic studies (Von Bothmer *et al.* 2003). The high diversity of barley phenotypes, true diploid nature, and ease of hybridization and cultivation has made barley species the favorite genetic organism. Moreover, mutations can more easily be identified in barley than in its polyploid relatives, such as wheat and oats (Scholtz *et al.* 2001). The reciprocal crossing analysis provides a unique opportunity to obtain a rapid and overall picture of the genetic control of a set of parents in the early generation (Crusio 1987). One of the symptoms of revolutionizing angiosperm molecular genomics is the development of new sets of genetic markers to genotype barley as long as other essential plant breeds (Yu *et al.* 2011). Several candidate markers are targeted on the level of DNA architecture, one of these candidate genes is chloroplast maturase K (*mat*K) gene. It was reported that *mat*K gene might be contributed to plant molecular systematics and evolution (Johnson & Soltis

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1995; Liang & Hilu 1996). The *mat*K gene, 1536 bp long, is located within the intron of the chloroplast gene *trn*K, on a large single-copy section adjacent to the inverted repeat (Hilu & Liang 1997). Maturases contain three domains: a reverse-transcriptase domain, domain X (the RNA binding and splicing domain), and a zinc-finger-like domain (Mohr *et al.* 1993; Barthet *et al.* 2015). It's reported that maturases are enzymes that catalyze non-autocatalytic intron removal from premature RNAs (Zoschke *et al.* 2010). Therefore, the *mat*K gene may be a strong candidate marker for evaluation of genetic polymorphism. The *mat*K gene with its underlying features represents a molecule that has substantial potential in providing insight into evolutionary and systematic problems at variable levels (Hilu & Liang 1997). Moreover, the ability to use *mat*K gene in the nucleotide polymorphism is reported (Barthet & Hilu 2007). However, very little genetic data is currently available about the *mat*K gene polymorphism in barley varieties around the world since no sufficient data that dealt with this hotspot genetic locus in *H. vulgare* varieties are available at least in terms of their genetic variability. Besides, no association study has highlighted this segment as a candidate genetic marker about its relationship with the main variable identities of *H. vulgare* variations. Thus, employing *mat*K, as a gene included in the chDNA in the genetic polymorphism could be considered as an interesting genetic marker. In crops, the detection of the single nucleotide polymorphisms (SNPs) permits a more accurate approach to the analysis of sequence differences between alleles (Rafalski 2002). To manifest this practically, several techniques are employed to exploit the portions of this gene to detect the extent of polymorphisms in a way that could be used in routine labs, such as SSCP. Although PCR-SSCP is not the only reliable technique to genotype breeds of different organisms and other available methods are used instead, its performance is one of the simplest and the most sensitive techniques in the detection of unknown DNA mutation (Gasser *et al.* 2006). Hence, the utilization for SSCP polymorphisms could lead to the finding of useful genetic markers of agricultural populations (Kuhn *et al.* 2008). Thus, much more attention is gained in the *mat*K gene to be used as an attractive tool to study the genetic polymorphism in this aspect. On the other hand, it is apparently demonstrated that many SNPs have functional effects on their corresponding protein structure especially by a single change in the amino acid (Liao & Lee 2010). There is an increasing awareness that other types of genetic variations can affect several aspects of protein expression and function. Synonymous SNPs, (sSNPs) or silent' mutations, are now widely acknowledged to be able to cause changes in protein expression, conformation, and function (Sauna & Kimchi-Sarfaty 2011). However, sSNPs do not alter amino acid sequences. Conversely, a non-synonymous single nucleotide polymorphism (nsSNP), which is present within the exon of a gene, is responsible for the incorporation of other amino acid and known to be one of the leading causes for the possible alteration of the encoded protein. However, tolerant nsSNPs are not deleterious and are not involved in any change, whereas deleterious nsSNPs have a profound influence on protein structure or its interaction (Pauline & Steven 2003). Therefore, it is important to differentiate deleterious nsSNPs from tolerant nsSNPs to characterize the genetic basis of these biochemical pathways. By using bioinformatics prediction tools, it would be possible to segregate neutral SNPs from SNPs of likely functional effect and could also be useful to explain the structural basis of the desired and undesired variations in matK protein characterizations. Thus, employing several computational tools in this gene could be considered as interesting genetic markers to give a perspective bioinformatics insight to the nature of the genotypes within this population. Therefore, two main objectives of this study are pursued. The first one is to see the potential participation of the *mat*K gene polymorphism in the reciprocal crossing between five different barley varieties. As well, to predict the potential functional effect of the observed nsSNPs and to speculate their possible influence on protein structure and function using several state-of-art *in silico* computational tools.

MATERIAL AND METHODS

Plants and experiment materials

The field study was conducted in Kurdistan region – Iraq, Qilyasan Agricultural Research Station,

Table 1

The primer BLAST system that is employed to cover most of *mat*K gene sequences by designing two pairs of primers

No.	Primer sequence	Position within *mat*K genbank accession number: AB078138	Amplicon length
1	FP: 5′- AGGGGTTTGCGATTGTTGTG -3′ RP: 5′- TGCCCCGATCCAGTACAAAA -3′	Start 302, Stop 321 Start 1169, Stop 1150	868 bp
2	FP: 5′- ACTGGATCGGGGCATCCTAT -3′ RP: 5′- CCGAACCCAATCGTTGCATA -3′	Start 1156, Stop 1175 Start 1384, Stop 1365	229 bp

Faculty of Agricultural Sciences, University of Sulaimani (35°34′307″ N, 45°21′992″ E and 765 masl), 2 km Northwest of Sulaimani city, during the autumn growing seasons 2010–2011(crossing between parents at first location only to produce the first filial at the three locations at 2011–2012). Five varieties and pedigrees of two-rowed *H. vulgare* were used in this study, which includes MORA, ABN, Arabi Aswad, Clipper, and Bohoth H1. These varieties were crossed in full diallel mating design to form 20 Artificial F1 hybrids (Online Suppl. Table 1). All the Artificial F1 hybrids along with their parents were grown in the following growing season. Seeds of 20 F1s with their 5 parents (25 entries) were sown in the field experiments; they were conducted in a randomized complete block design (RCBD) with three replications. Each treatment was one row of 2-meter length, 40 cm between rows and 20 cm between plants within a row. The major features of these five varieties were mentioned in concomitance with their main sources (Online Suppl. Table 2).

DNA Samples

Total genomic DNA was obtained from twenty-five varieties (parents and artificial F1 hybrids) of barley seeds. DNA was isolated from about 25 mg seed using genomic DNA mini kit – Plant (Cat # GP100, Geneaid Biotech – Taiwan) following manufacturer's instructions. The purity of DNA was assessed by NanoDrop spectrophotometer, version BioDrop µLITE (Biodrop – UK). The integrity of isolated DNA was checked by ethidium bromide (0.5 mg/ml) pre-stained 0.8% (w/v) agarose gel electrophoresis in 1X TAE (40 mM Tris-acetate; 2 mM EDTA, pH 8.3) buffer. The isolated DNA was used as a template for PCR.

PCR Primers Designing and Amplification

Two fragments were designed for the *mat*K gene (1536 bp length) from GenBank accession number: AB078138 of NCBI website (www.ncbi.nlm.nih.gov), using Primer^3 online software (http://www.www.simgene.com/Primers). These two fragments cover most of the *mat*K gene (Table 1). The lyophilized primers were purchased from Bioneer (Bioneer, Daejeon, South Korea). PCR reaction was performed using the *AccuPower* PCR premix (Cat # K-2012, Bioneer – South Korea). Each 20µl of PCR premix was contained 1 U of *Top* DNA polymerase, 250 µM of four dNTPs, 10 mM of Tris-HCl (pH 9.0), 30 mM of KCl, 1.5 mM of $MgCl_2$. The PCR reaction mixture was completed with 10 pmol of each primer and 50 ng of genomic DNA. The following program was applied in gradient PCR thermocycler (Mastercycler-nexus, Eppendorf, 22331Hamburg). The optimum annealing temperature for both designed PCR amplicons was determined by gradient PCR. Then, the amplification was began by initial denaturation (94°C for 5 min), followed by 30 cycles of denaturation (94°C for 30 sec), annealing (52°C for 30 sec), and elongation (72°C for 30 sec), and was finalized with a final extension (72°C for 10 min). Amplification was verified by electrophoresis on an ethidium bromide (0.5 mg/ml) pre-stained 1.5% (w/v) agarose gel in 1 × TBE buffer (2 mM of EDTA, 90 mM of Tris-Borate, pH 8.3). It was made sure that all PCR resolved bands were distinct and consisted of only one clean and sharp band to be submitted into SSCP successfully (Online Suppl. Figure 1).

SSCP analysis

The SSCP experiments were initially based on Al-Shuhaib SSCP protocol (Al-Shuhaib 2017), with some modifications. Different gel concentrations, sample volumes, electrophoresis temperatures were tested to optimize separation power of SSCP experiments. Briefly, 7 µl of each amplification product was mixed with 10µl of SSCP denaturing loading buffer (95% formamide, 20 mM EDTA pH 8, 0.05% xylene cyanol and 0.05% bromophenol blue). The samples were heat-denatured at 95°C for 10 min and chilled on ice for at least 5 min, and loaded polyacrylamide gel. The preliminary SSCP experiments were performed in the commercially available mini gels (10 × 10 × 1) mm version; OminiPage (Cleaver Scientific – UK). After optimization, PCR amplicons were separated onto mini-high resolution aluminum plates vertical gel format, gel size (W × L) cm: 12 × 14.5, and gel thickness: 1 mm (model JY-SCZ9, Junyi-Dongfang Electrophoresis Equipment – China). Denatured PCR products were loaded into the wells of 8% acrylamide/bis (37.5:1), containing 7% glycerol, and 1X TBE buffer. The gel was run under 225 V / 112 mA at room temperature until the tracking dye reached the end of the gel. Each SSCP gel was stained by a rapid silver staining technique (Byun *et al.* 2009).

DNA sequencing and sequencing analysis

Each template DNA sample that showed a different pattern on the SSCP gel for the amplified *mat*K gene fragments was sequenced from both ends (Macrogen Inc. Geumchen, Seoul, South Korea). Only clear chromatographs obtained from ABI sequence files were further analyzed, ensuring that the annotation and variations are not because of PCR or sequencing artifacts. The referring *Hordeum vulgare mat*K database (GenBank acc. AB078138.1) was retrieved from the NCBI website (https://www.ncbi.nlm.nih.gov/nuccore/AB078138.1). The sequencing results of the PCR products of different SSCP patterns were edited, aligned, and analyzed as long as with the respective sequences in the reference database using *BioEdit* Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA).

Constructing the primary structure of the altered matK protein

The primary protein structure building of each SSCP genotype was begun by mutating the available reference NCBI DNA sequences of the *mat*K gene, by substituting each observed SNP from the *BioEdit* /Lasergene software into its accurate position in the whole reference the *mat*K DNA sequence to represent each altered variant. The observed variation was translated into amino acids in a reading frame corresponds to the reference matK amino acid sequences using the *Expasy* online program (http://web.expasy.org/translate/). Multiple amino acid sequence alignment was made between the reference matK amino acid sequences and its observed variations using the Clustal Omega program from the *UniProt* website (http://www.uniprot.org/align/).

Finding the deleterious effect of the matK protein nsSNP using SIFT

The amino acid sequence of matK along with non-synonymous SNPs (nsSNPs) with corresponding amino acid positions was submitted using SIFT (sorting intolerant from tolerant) program (Pauline & Steven 2003). The SIFT prediction tolerance index (TI) score was ranged from 0.0 to 1.0, which denoted the normalized probability that the amino acid change was tolerated. Substitutions at each position with normalized probabilities less than a tolerance index of 0.05 were predicted to be deleterious or intolerant; those greater than or equal to 0.05 were predicted to be tolerated (http://sift.bii.a-star.edu.sg/www/SIFT_seq_submit2.html).

Predicting the functional effect of nsSNPs using PolyPhen-2

PolyPhen or Polymorphism Phenotyping is a tool which predicts possible impact of an amino acid substitution on the structure and function of the protein using straightforward physical and comparative considerations. PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), is a new development of the PolyPhen tool for annotating coding nonsynonymous SNPs (Adzhubei *et al.* 2010). Prediction outcomes could be classified as probably damaging or benign according to the score ranging from 0–1 respectively.

Prediction of functional impact of the matK nsSNP using PROVEAN

Further confirmation of the effect of nsSNPs on protein was done using PROVEAN (Protein Variation Effect Analyzer) tool. PROVEAN tool can predict the impact of an amino acid substitution on the biological function of the protein (http://provean.jcvi.org/index.php). The default threshold of PROVEAN tool is –2.5, *i.e.*, variants with a score equal to or below –2.5 are considered “deleterious,” while variants with a score above –2.5 are considered "neutral" (Choi *et al.* 2012).

Prediction of the severity effect of the matK nsSNP using SNAP2

SNAP2 is a tool that can distinguish between the effect and the neutral nsSNPs (Smigielski *et al.* 2000). Predicting a score (ranges from −100 strong neutral prediction to +100 strong effect prediction), the analysis prediction scores were potentially correlated with the severity of effect (https://www.predictprotein.org).

Investigation of altered protein stability of the matK nsSNPs using I-Mutant 2.0

To have a better insight to the stability of the protein caused by mutation, the altered positions were analyzed using I-Mutant 2.0 (Capriotti *et al.* 2005). The study of the effect of a mutation may alter the stability of the matK protein of interest and might lead to a change in the main characteristics of this protein. I-Mutant 2.0 (http://folding.biofold.org/cgi-bin/i-mutant2.0) is a tool for the prediction of protein stability changes upon missense mutations.

Three-dimensional matK structure modeling using RaptorX and PyMol

The whole amino acid sequences of barley matK were retrieved from NCBI (GenBank acc. No. AB078138.1). The UniProtKB/Swiss-Prot accession number for barley matK protein was P17158, and no matching PDB entries were found in this protein (http://www.uniprot.org/uniprot/P17158). The three-dimensional (3-D) structure of the *mat*K gene was constructed from the online 3-D model prediction software; namely RaptorX (http://raptorx.uchicago.edu/StructurePrediction/predict/). The observed mutations were inserted in the native sequence of the entire 511 amino acid residues matK protein to represent the observed variation. Then, the 3-D structures of the native matK protein, as well as the observed alteration were predicted using RaptorX tool. For given FASTA query sequence, RaptorX predicted its secondary and tertiary structures as well as solvent accessibility and disordered regions (Kallberg *et al.* 2012). The virtually proposed changes within its corresponding altered proteins were performed by using PyMol-v1, 7.0.1 software (www.shrodinger.com).

Prediction of some physio-chemical properties of the matK protein

A novel META-Disorder prediction method, or MD tool, molds various sources of information predominantly obtained from orthogonal prediction methods, to significantly improve in performance over its constituents (Schlessinger *et al.* 2009). Profisis tool, which is a web server that can predict protein-protein and protein – polynucleotide binding sites, was utilized to identify the interacting residues from sequence alone (Ofran & Rost 2007). Both tools that utilized for these predictions were available on (https://www.predictprotein.org/).

Post-translation modification sites present on matK protein

Glycation sites of ε amino groups of lysine residues were predicted using a NetGlycate1.0 server (http://www.cbs.dtu.dk/services/NetGlycate/). In NetGlycate a score of N0.5 was considered glycated. Phosphorylation sites were predicted using a NetPhos3.0 server (http://www.cbs.dtu.dk/services/ NetPhos/). In NetPhos3.0, serine, threonine, and tyrosine residues with a score of N0.5 were considered phosphorylated. Ubiquitylation sites were predicted using UbPerd (www.ubpred.org). In UbPerd, lysine residues with a score of ≥0.62 were considered ubiquitylated. Sumoylation sites were predicted using SUMOplot (http://www.abgent.com/sumoplot). For SUMOplot, high probability motifs having a score of 0.5 were considered sumoylated (Jenna & Stephen 2014).

RESULTS

To identify the reason that lies behind the variations of barely, it was deduced to study the chloroplast matK protein as a possible candidate in this regard. Accordingly, we have artificially created variable entries of barley in this study to see – if any – possible participation of the *mat*K gene in this reciprocal crossing between five different parental varieties (Figure 1, A). By designing two specific PCR primers pairs, 390 out of 511 amino acid residues were covered, which constitute about 76% of the whole amino acid sequences of the chloroplast matK protein in twenty-five artificially crossed *H.*

vulgare varieties. The genetic polymorphism of each observed haplotypes was predicted computationally. Two sets of results were obtained in this study; *in vitro* genotyping results through PCR-SSCP-sequencing experiments, and *in silico* prediction results through several bioinformatics tools.

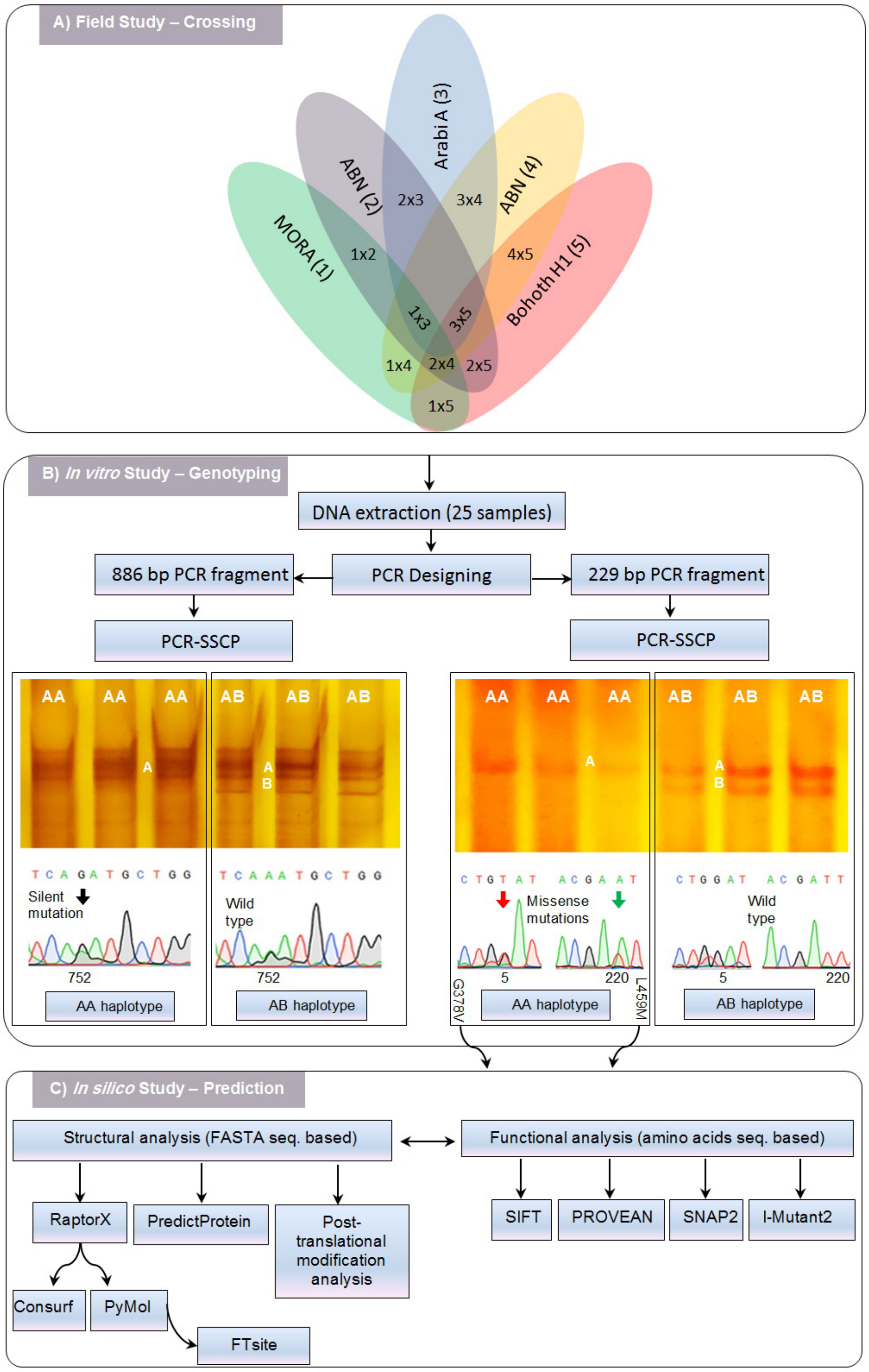

Figure 1. A full schematic workflow of the *mat*K gene polymorphism study. This study consists of three main partitions; A) field study (crossing of the five different parents), B) *in vitro* genotyping through PCR-SSCP-sequencing of both designed amplicons of 868 bp and 229 bp, in which two genotypes were resolved in each case, and C) *in silico* computational analysis of the observed missense mutations.

Table 2

Nucleotide substitutions and types among haplotype A, and haplotype B, and the reference NCBI sequence of *H. vulgare* *mat*K gene

The size of amplicon	Nucleotide position in the NCBI reference *mat*K gene	Nucleotide position in the PCR fragment	SSCP Haplotypes of *Hordeum vulgare* *mat*K gene			Amino acid change	Type of SNPs
			Reference	A haplotype	B haplotype		
868 bp PCR fragment	1053	752	A	G	–	non	Synonymous
*mat*K 229 bp PCR fragment	1160	5	G	T	–	G387V	Non-synonymous
	1375	220	T	A	–	L459M	Non-synonymous

Genotyping analysis

The polymorphism of *mat*K gene was detected by PCR-SSCP and DNA sequencing methods, and the observed variations of SSCP gels indicate the detection of two different variations. In both studied PCR fragments, two haplotypes were resolved, AA and AB (Figure 1, B). The pattern and nature of each SNP that was detected by sequencing indicated that the AA variation has one novel sSNP (A1053G), while in the variation AB no SNP was observed (Table 2). The haplotype distribution is slightly predominated by AB haplotype. Despite the fact that the multitude of SSCP bands that we observed in this studied *mat*K gene fragment, this is usually evident in some SSCP configurations that exceed 600 bp (Gasser *et al.* 2006). However, both haplotypes were available in both parents and offspring, suggesting a nonparticipation of *mat*K large gene fragment in the reciprocal crossing that performed between parents. In this aspect, the situation was the same in *mat*K the 229 bp gene fragment, in which two haplotypes were revealed. It was revealed that the haplotype AA has much more frequencies over variant AB. The variant AA was the predominant one since the number of variant AA in this study was fifteen samples in both parents and offspring, which constituted about 60%, while the variant AB was less common since it was included only ten samples, whereas no other samples showed any other noticeable variant even after repeating SSCP electrophoresis for several times. Sequencing results confirmed these two different electrophoretic SSCP patterns since two SNPs were detected between the two resolved variations and NCBI reference sequences. Both nsSNPs, (G1160T and T1375A), were found in AA variants. However, the functional nsSNPs were apparently shown only in the 229 bp PCR fragment out of the whole matK amino acid sequences. According to PCR-SSCP-Sequencing, two conformational patterns of both *mat*K PCR fragments were observed in this study as they determined in parental and artificial F1 barley samples. However, it was revealed that both variants were the only genetic polymorphisms that were available for both parents and their artificial F1 hybrids.

Computational analysis

To get better exploration to the detailed genetic variation(s) within the *mat*K gene, both SSCP-DNA sequencing variations of both PCR fragments were computationally analyzed by several *in silico* tools (Figure 1, C). While only one non-coding SNP is positioned in the AA variant of the 868 bp PCR fragment, two coding SNPs that were available only in the AA variants of the 229 bp PCR fragment. In both nsSNPs, the hydrophobic aliphatic amino acids Gly and Leu in the positions 387 and 459 of the whole matK proteuren (Figure 2, A) are substituted into another amino acids of the same group, which is Val and Glu respectively (Figure 2, B). The consequences of these nsSNPs were evaluated using different publicly available computational algorithms, namely, SIFT, PolyPhen-2, SNAP2, PROVEAN, and I-Mutant 2 bioinformatics tools (Table 3). However, by comparing the prediction of these methods for each observed nsSNP, variable results were observed. Usually, the SIFT tool is utilized first, which predicts whether an amino acid substitution affects protein function among related genes and domains over evolutionary time (Ng & Henickoff 2006). SIFT predicts that G387V nsSNP was found to be tolerated, which showed a highly acceptable tolerance index score (more than 0.05), which couldn't affect the protein function. While in the case of L459M, SIFT predicts an effect on protein function, which showed an unacceptable

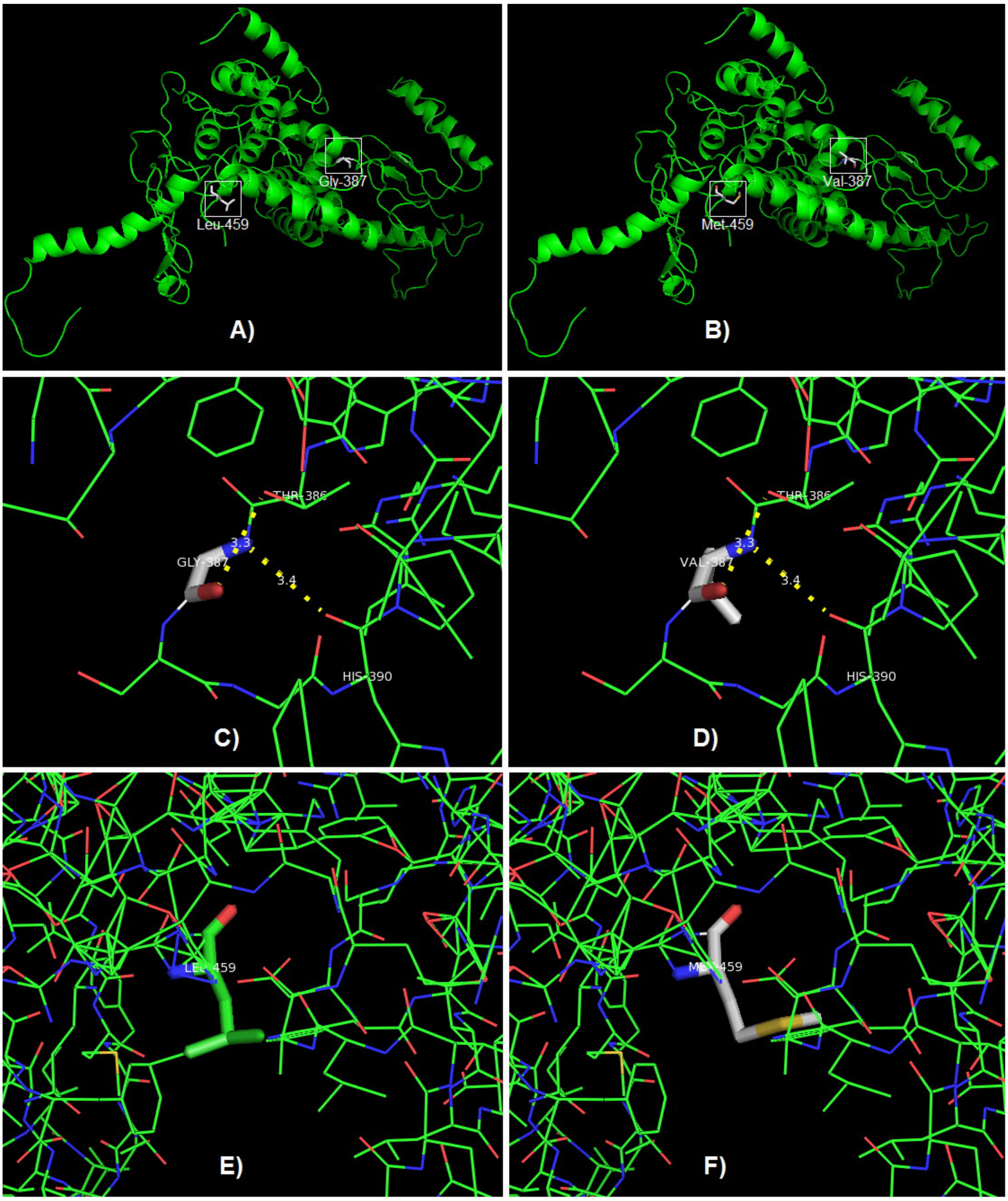

Figure 2. The proposed 3-D structure of matK protein of *H. vulgare* species with its polar interactions. A) The native protein is shown with two highlighted amino acids (G387 and L459). B) The altered protein is shown with two altered amino acids (V387 and M459). C) The interaction of S387 with both T386 and H390 before mutation. D) The interaction of V387 with both T386 and H390 after mutation. E) The absence of any polar connections in the L387 before mutation. F) The absence of any polar connections in the M387 after mutation.

Table 3

List of the possible effects and consequences of the observed nsSNPs on matK protein structure and function as determined by four bioinformatics tools

nsSNP	SIFT	PolyPhen-2	PROVEAN	SNAP2	I-Mutant 2.0	
G387V	1	0.002 (sensitivity: 0.99) (specificity: 0.30)	4.470 $^{\text{CF}*}$	–50	–1.22$^{\text{DDG}*}$ (reliability index: 3)	Score
	Tolerated	Benign	Neutral	Neutral	Decrease	Prediction
L459M	0.00 $^{\text{LC}*}$	0.98 (sensitivity: 0.75) (specificity: 0.96)	–0.730 $^{\text{CF}*}$	–21	–1.14$^{\text{DDG}*}$ (reliability index: 8)	Score
	Affect protein function	Probably damaging	Neutral	Neutral	Decrease	Prediction

$^{\text{CF}*}$; cutoff = –2.5, $^{\text{LC}*}$: (LC; low confidence); this substitution may have been predicted to affect function just because the sequences used were not diverse enough. $^{\text{RI}*}$; reliability index score. $^{\text{DDG}*}$; DG(NewProtein)-DG(WildType) in Kcal/mol. $^{\text{S,S}*}$; (Sensitivity: 0.99, Specificity: 0.30)

tolerance index score (less than 0.05), which could affect the protein function. However, the last one SIFT prediction had little confidence because the submitted *H. vulgare* matK protein sequence wasn't diverse enough for this program to give high confidence prediction. To predict the possible impact of these nsSNPs on both structure and function of the matK protein, the PolyPhen-2 program was used (Adzhubei *et al.* 2010). In agreement with SIFT results, PolyPhen-2 has predicted the same outcomes of these two SNPs. Thus, based on both SIFT and PolyPhen-2, the missense mutation L459M would be of prime importance concerning both its structural and functional effect on the matK protein. The SIFT/ PolyPhen-2 predicted characterization of G387V, and L459M nsSNPs were further validated using PROVEAN tool (Choi *et al.* 2012). The default threshold of PROVEAN tool is –2.5, *i.e.*, variants with a score equal to or below –2.5 are considered "deleterious", while variants with a score above –2.5 are considered "neutral". In contrast to SIFT/ PolyPhen-2 variable scores, PROVEAN revealed that both these nsSNPs weren't deleterious. It predicts that G387V and L459M nsSNPS are neutral (below –2.5). The SNAP2 program was also used to assess the effect of both SNPs on the matK protein sequence. In agreement with PROVEAN program, SNAP2 was found that both G387V and L459M were neutral (less than 0). Nevertheless, in the last two programs, there is a slight tendency of L459M to be more effective. However, it's interesting to note that when these four programs were used together, we observed 100% agreement of the SIFT/ PolyPhen-2/PROVEAN/SNAP2 results and 50% of agreement of these programs on the G387V program. To add another layer of confirmation, these two variants were submitted to the I-Mutant-2.0 web server to predict the DDG stability and reliability index (RI) upon mutation. If the DDG value is >0, protein stability decreases and when DDG value is <0 protein stability increases. The I-Mutant 2.0 program showed a reduced stability of the matK protein for both two nsSNPs. Thus, there are higher chances that protein stability might get affected in mutation at two amino acid positions; 378 (DDG score –1.22), and 459 (DDG score –1.14).

The 3-D structure of the native matK model that generated through RaptorX (Kallberg *et al.* 2012) was revealed that all 511 (100%) residues were modeled, and only 1 (0%) positions were predicted as disordered, while the mutated protein has 25 (4%) positions were predicted as disordered. Secondary structures of the native matK model revealed 50% helix, 9% beta sheet, and 40% loop structures, while the secondary structure of the altered protein model showed 42% helix, 10% beta sheet, and 47% loop structures. The protein solvent accessibility was divided into three states by 2 cut-off values: 10% and 42%. A value less than 10% was identified as buried, larger than 42% value was identified as exposed and if the value was between 10% and 42% was designated as a medium. Regarding solvent access, noticeable differences were found between native and altered protein. The proportions of exposed, medium and buried regions in the native matK protein were 37%, 15%, and 47% respectively, while in the altered protein was 31%, 37%, and 30% respectively. The nature of the polar interaction of the observed three nsSNPs was tested by PyMol to unravel its possible role in the conversion of the nature of the native amino acid binding with its surrounding residues in the matK protein.

It was found that G387 in the native protein has two polar interactions with Thr386 and His390 of 3.3 Å and 3.4 Å lengths respectively (Figure 2, C). While the altered amino acid 387V forms the same polar interactions with the same residues and distances (Figure 2, D). In the case of L459M nsSNP, it was found that L459 in the native protein doesn't have any polar interactions (Figure 2, E); and the situation is the same for the altered 459M (Figure 2, F).

It is necessary to identify the disordered regions in proteins from their amino acid sequences since

Table 4

Protein disorder possibility prediction of the S387V and L459M nsSNPs in the matK protein

Number	Residue	NORSnet	NORS2st	PROFbval	Ucon	Ucon2st	MD_raw	MD_rel	MD2st
387	G	0.31	–	0.55	D	0.19	–	0.263	8
459	L	0.08	–	0.55	D	0.14	–	0.263	8

Number – residue number; Residue – amino acid type; NORSnet – raw score by NORSnet (prediction of unstructured loops) NORS2st – two-state prediction by NORSnet; D = disordered; PROFbval – raw score by PROFbval (prediction of residue flexibility from sequence); Bval2st – two-state prediction by PROFbval; Ucon – raw score by Ucon (prediction of protein disorder using predicted internal contacts); Ucon2st – two-state prediction by Ucon; MD_raw score by MD (prediction of protein disorder using orthogonal sources); MD_rel – reliability of the prediction by MD; values range from 0–9. 9 = strong prediction; MD2st – two-state prediction by MD

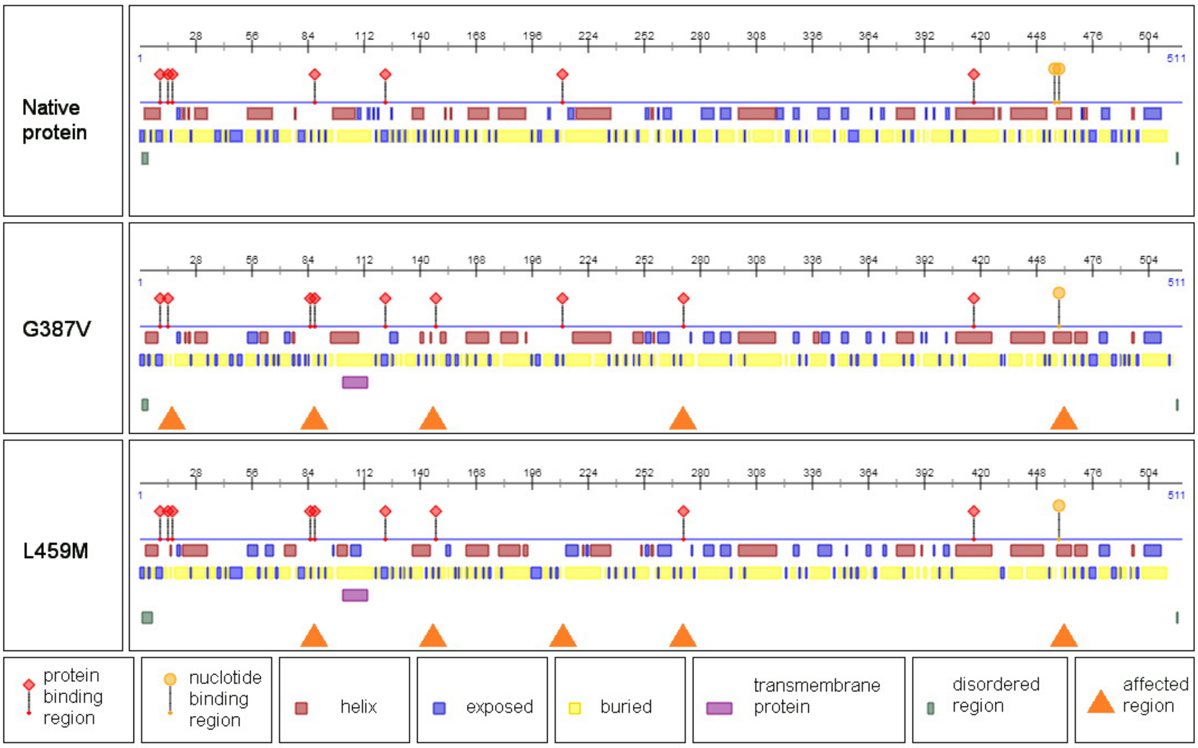

Figure 3. Prediction of protein-protein and protein-polynucleotides binding sites for native matK protein A), and its altered G387V mutant B), and L459M mutant proteins C)

the disordered regions are also important for the functional annotation of proteins. The available prediction MD tool has enabled us to use the "meta approach" to predict disordered regions (Schlessinger *et al.* 2009). Interestingly, MD program predicted that both of G387V and L459M occupied readily disordered positions in the native matK protein (Table 4). Since the disordered regions that have no stable structures without their partner molecules are often found in functional sites of proteins (Ward *et al.* 2004), a potential participation of these two nsSNPs in the binding with other proteins, such as transcription factors or polynucleotide binding proteins was predicted. This prediction was confirmed using profISIS, which has shown noticeable differences between the native and the altered matK protein. In comparison to the native protein, 4 protein binding sites were altered in the mutant G37V and L459M proteins. Besides, one additional polynucleotide binding site and a transmembrane protein were observed (Figure 3). This observation indicates the possible interaction of G387V and L459M with the binding with other proteins and polynucleotides. Several *in silico* post-translational modification prediction tools, such as NetGlycate1.0, NetPhos3.0, UbPerd, and SUMOplot were utilized to identify the potential participation of G387V and L459M in the post-translational modification. No participation was observed for both nsSNPs in all studied post-translational glycation, phosphorylation, ubiquitylation, and sumoylation modification sites (Online Suppl. Table 3).

DISCUSSION

Initially, we were led to engage the *mat*K gene polymorphism in this study because of several reasons; 1) The chloroplast *mat*K DNA sequence, a chloroplast-encoded locus that has been reported to relatively have higher substitution rates at both nucleotide and amino acid level than other genetic loci (Barthet *et al.* 2007). 2) The *mat*K gene has recently emerged as an invaluable gene in plant systematics because of the high phylogenetic signal it has compared with other genes used in this field (Muller *et al.* 2006). 3) Its protein is the only candidate for splicing introns from many genes in chloroplast (Zoschke *et al.* 2010). 4) Its worth mentioning that there has not been any sufficient data regarding the effect of *mat*K genetic polymorphisms on the protein structure. Thus, for its essential and irreplaceable function in the plant makes the *mat*K gene as an excellent marker that may deserve to deeply explored in genetic breeding.

Genotyping analysis

The sequencing that performed in both 686 bp and 229 bp *mat*K gene fragments were confirmed the discrete genetic differences between them in the SSCP gel. These SNPs are novel and have not been previously reported in the *mat*K gene of *H. vulgare* (https://www.ncbi.nlm.nih.gov/projects/SNP/). It deserves to note that there is no significant relationship between the identified SNPs patterns in *mat*K gene and diallel and reciprocal crossing that performed among five different parental varieties. Although the SSCP results of *mat*K 686 bp and 229 bp gene fragments are shown significant variations in barley varieties, no obvious consistent results were obtained regarding the pattern of crossing among these varieties. Nonetheless, distinct variable forms of polymorphisms were obtained in parents concerning both *mat*K gene PCR amplified fragments. These distinct SSCP results might be referred to the presence of a wide ratio of hybridization, selection, or genetic diversity for barley (Schneider *et al.* 2001). The presence of the polymorphism in the *mat*K gene does not imply that this gene has a role in the variations among the studied diallel or reciprocal crossed five varieties. This is evident since we found that both genetic varieties existed in both parental and F1 hybrids varieties. Therefore, the SNPs detected here may not be a causal mutation. Although no comparable standards were available in these local varieties, the results of this study in the barley samples have yielded an initial molecular marker that can be applied to identify the sort of polymorphism in other nearby barley samples, through relying on the amplification of both 686 bp and 229 bp *mat*K gene PCR fragments. However, it is evident from this study that the use of 229 bp PCR *mat*K gene fragment might be more informative than the 686 bp PCR fragment *mat*K gene as the two observed nsSNPs of this study is readily detected using this fragment.

Computational analysis

The dramatic revolution in the computational prediction of the individual nsSNPs made it possible to generate an overview for the final destination for each nsSNP on the protein structure and function (Patel *et al.* 2015). As long as *in vitro* genotyping analysis, we have utilized *in silico* prediction tools to get a final estimation of the possible role of each observed nsSNP on the modulating of the main characteristic features of the matK protein. Three SNPs were discovered in this study; one is sSNP, two are nsSNPs. It was found through several computational tools that the effect of these SNPs is ranged from non-effective into mild-effective on protein structure and function. As it was known, the understanding the roles of some of the nsSNPs can significantly help to understand the barley genotype/phenotype variations. We opted to use *in silico* tools based on a combination of different algorithms that previously reported for other genes for the analysis of the observed amino acid variations in the *mat*K gene. As genomic variations view among barley is common sense (Hilu & Liang 1997), the nsSNPs that exist through the coding region of this genome can, therefore, become necessary since they modulate the amino acid composition (Liao & Lee 2010). Such changes can have an impact on protein structure, function, stability, and subcellular localization. The nsSNPs may initiate unique structures between population members in conferring different protein biological activities. Likewise, nsSNPs may affect the *mat*K gene expression by inactivating the active sites of the matK protein through which it interacts with the exon-intron junctions during splicing. Therefore, the observed nsSNPs was given special attention in this study. To determine the functional effect of these two nsSNPs on matK protein, several widely used *in silico* tools were employed in this study, specifically SIFT, PolyPhen-2, SNAP2, PROVEAN, and I-Mutant 2.0 (Pauline & Steven 2003; Capriotti *et al.* 2005; Adzhubei *et al.* 2010; Smigielski *et al.* 2000; Choi *et al.* 2012) respectively. However, the utilized bioinformatics tools had given different predictions on the deleterious effect of both nsSNPs on the matK protein. The differences in the results of these several prediction tools are due to the differences in features utilized by the methods; therefore we would expect the outcomes to occur dissimilar at some point (De Alencar *et al.* 2010). SIFT, PolyPhen-2, SNAP2, and PROVEAN tool had all given parallel results about the non-effectiveness of S387V on the matK protein function. Meanwhile, SIFT and PolyPhen-2 showed that L459M was effective and damaging, whereas both SNAP2 and PROVEAN had shown a neutral effect of this nsSNP. However, taking these notes into account, L459M is an effective missense mutation in comparison with S387V. Concerning the stability of the altered protein, I-Mutant 2.0 showed a decrease in stability for these two nsSNPs upon mutation. Thus, data this suggests that S387V and L459M variants of the *mat*K gene could directly or indirectly modulate the amino acid interactions causing functional deviations of protein to some extent. In addition to the previously mentioned bioinformatics tools, the polar interaction of these nsSNPs with other residues was shown no modification to the binding pattern with other residues. This suggestion was validated through MD prediction tool (Schlessinger *et al.* 2009). It is important to identify the disordered or the ordered regions from their amino acid sequences since the prediction of such configuration is also important for the functional annotation of proteins. In addition to the involvement in many biological processes, such as transcription, translation, signaling and cell cycle control, the disordered regions are also involved in alternative splicing (Romero *et al.* 2006). Hence, this finding may have some effect on the matK protein splicing mechanism. Upon binding with ligands, disorder-to-order transitions are frequently observed, where the flexibility of the disordered regions may be necessary to specifically interact with multiple partners (Ishida & Kinoshita 2007). Though no internal amino acids connection were changed according to PyMol prediction, the differences observed between the native and altered matK protein in the external binding with other proteins and polynucleotides as determined by ISIS program may be reflected in its function in the splicing of exon-intron junctions too (Zoschke *et al.* 2010), which may lead to a change in the genetic expression. This observation may become evident during splicing when the matK protein domain X binds to intron to form a lariat structure. It does remain bound to the excised RNA to form a ribonucleoprotein particle

(Saldanha *et al.* 1999). The formation of this particle may be modulated as a result of the role of these two nsSNPs in the alteration of the binding of the matK protein with polynucleotides. Though the precise mechanism by which matK protein potentially interacts with other factors still not fully understood, this finding may pinpoint some initial data which may help in the accurate understanding of these motifs which may change the substrates with which they are interacting. Eventually, the bioinformatics tools helped us to characterize the impact of nsSNP on the *mat*K gene and suggest that the *in silico* analysis may be a useful tool to predict the effect of DNA variation on gene function.

Eventually, it had been hoped that the genotyping analysis would exploit some sections of the genome which are highly variable, such as the *mat*K gene, to be used for varieties differentiation particularly in diallel and reciprocal crossing. Nonetheless, this genetic segment doesn't differentiate between the different varieties since we have chosen a region that despite its confirmed variability it might not has a relation with the pattern of the genealogical variations among these diallel or reciprocal crossed varieties. Although the two resolved variants may not be connected with the nature of the crossed varieties, both *mat*K amplified fragments have exerted only two distinct variants in all of these varieties. This study has computationally found a noticeable reduction in the matK protein stability upon mutation with both S378V and L459M. In addition to MD/ISIS prediction, a concomitant alteration in the binding with the external proteins and polynucleotides were predicted in these two observed nsSNPs too.

CONCLUSIONS

This study predicts that both S378V and L459M missense mutations of the *mat*K gene may participate in the alteration of the matK protein splicing of intron-exon splicing mechanisms. This pilot study may determine the pattern of polymorphism within the *mat*K gene to conceive its consequences within these highly important barley varieties.

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the onlive wersion, at doi: 10.2478/agri-2018-0001

REFERENCES

ADZHUBEI, I.A. – SCHMIDT, S. – PESHKIN, L. – RAMENSKY, V.E. – GERASIMOVA, A. – BORK, P. – KONDRASHOV, A.S. – SUNYAEV, SR. 2010. A method and server for predicting damaging missense mutations. In *Nature Methods*, vol. *7*, no. 4, pp. 248–249. DOI: 10.1038/nmeth0410-248

AL-SHUHAIB, M.B. 2017. A Universal, rapid, and inexpensive method for genomic DNA isolation from the whole blood of mammals and birds. In *Journal of Genetics*, vol. *96*, no 1, pp. 171–176. DOI: 10.1007/s12041-017-0750-6

BARTHET, M.M. – HILU, K.W. 2007. Expression of matK: functional and evolutionary implications. In *American Journal of Botany*, vol. *94*, no. 8, pp. 1402–1412. DOI: 10.3732/ajb.94.8.1402

BARTHET, M.M. – MOUKARZEL, K. – SMITH, K.N. – PATEL, J. – HILU, K.W. 2015. Alternative translation initiation codons for the plastid maturase MatK: unraveling the pseudogene misconception in the Orchidaceae. In *BMC Evolutionary Biology*, vol. *15*, pp. 210. DOI: 10.1186/s12862-015-0491-1

BYUN, S. – FANG, Q. – ZHOU, H. – HICKFORD, J. 2009. An effective method for silver-staining DNA in large numbers of polyacrylamide gels. In *Analytical Biochemistry*, vol. *385*, pp. 174–175. DOI: 10.1016/j.ab.2008.10.024

CAPRIOTTI, E. – FARISELLI, P. – CASADIO, R. 2005. I-Mutant 2.0: predicting stability changes upon mutation from the protein sequence or structure. In *Nucleic Acids Research* vol. *33*, pp. 306–310. DOI: 10.1093/nar/gki375

CHOI, Y. – SIMS, G.E. – MURPHY, S. – MILLER, J.R. – CHAN, A.P. 2012. Predicting the functional effect of amino acid substitutions and indels. In *PLOS ONE*, vol. *7*, pp. e46688. DOI: 10.1371/journal.pone.0046688

CRUSIO, W. 1987. A note on the analysis of reciprocal effects in diallel crosses. In *Journal of Genetics*, vol. *66*, pp. 177–185. DOI: 10.1007/BF02927711

DE ALENCAR, S.A. – LOPES, J.C.D. 2010. A comprehensive in silico analysis of the functional and structural impact of SNPs in the IGF1R gene. In *Journal of Biomedical Biotechnology* ID 715139, 8 pages. DOI:10.1155/2010/715139

GASSER, R.B. – HU, M. – CHILTON, N.B. – CAMPBELL, B.E. – JEX, A.J. – ONTRANTO, D. – CAFARCHIA, C. – BEVERIDGE, I. – ZHU, X. 2006. Single-strand conformation polymorphism (SSCP) for the analysis of genetic variation. In *Nature Protocols*, vol. *1*, no. 8, pp. 3121–8. DOI:10.1038/nprot.2006.485

HILU, K.W. – LIANG, H. 1997. The *mat*K gene: sequence variation and application in plant systematics. In *American Journal of Botany*, vol. *84*, pp. 830–839. url: https://www.ncbi.nlm.nih.gov/pubmed/21708635

ISHIDA, T. – KINOSHITA, K. 2007. PrDOS: prediction of disordered protein regions from amino acid sequence. In *Nucleic Acids Research*, vol. *35*, pp. W460–464. DOI: 10.1093/nar/gkm363

JENNA, N.K. – STEPHEN, D.B. 2014. In silico analysis of functional single nucleotide polymorphisms in the human TRIM22 gene. In *PLoS One*, vol. *9*, no. 7, pp. e101436. DOI: 10.1371/journal.pone.0101436

JOHNSON, L.A, – SOLTIS, D.E. 1995. Phylogenetic inference in saxifragaceae sensu stricto and gilia (Polemoniaceae) using matK sequences. In *Annals of the Missouri Botanical*

Garden, vol. *82*, pp.149–175. DOI: 10.2307/2399875

KALLBERG, M. – WANG, H. – WANG, S. – PENG, J. – WANG, Z. – LU, H. – XU, J. 2012. Template-based protein structure modeling using the RaptorX web server. In *Nature Protocols*, vol. *7*, no. 8, pp. 1511–1522. DOI: 10.1038/nprot.2012.085

KUHN, D. – MOTAMAYOR, J. – MEEROW, A. – BORRONE, J. – SCHNELL, R. 2008. SSCP markers provide a useful alternative to microsatellites in genotyping and estimating genetic diversity in populations and germplasm collections of plant specialty crops. In *Electrophoresis,* vol. *29*, no. 19, pp. 4096–5108. DOI: 10.1002/elps.200700937

LIANG, H. – HILU, K.W. 1996. Application of the *mat*K gene sequences to grass systematics. In *Canadian Journal of Botany,* vol. *74*, pp. 125–134. url: http://www.nrcresearchpress.com/doi/pdf/10.1139/b96-017

LIAO, P.Y. – LEE, K.H. 2010. From SNPs to functional polymorphism: The insight into biotechnology applications. In *Biochemical Engineering Journal*, vol. *49*, pp. 149–158. https://doi.org/10.1016/j.bej.2009.12.021

MOHR, G. – PERLMAN, P. – LAMBOWITZ, A. 1993. Evolutionary relationships among group II intron-encoded proteins and identification of a conserved domain that may be related to maturase function. In *Nucleic Acids Research*, vol. *21*, pp. 4991–4997. url: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC310608/

MULLER, K.F. – BORSCH, T. – HILU, K.W. 2006. Phylogenetic utility of rapidly evolving DNA at high taxonomical levels: contrasting *mat*K, trnT-F and rbcL in basal angiosperms. In *Molecular Phylogenetics and Evolution,* vol. *41*, pp. 99–117. DOI: 10.1016/j.ympev.2006.06.017

NG, P.C. – HENICKOFF, S. 2006. Predicting the effects of amino acid substitutions on protein function. In *Annual Review of Genomics and Human Genetics*, vol. *7*, pp. 61–80. DOI: 10.1146/annurev.genom.7.080505.115630

OFRAN, Y. – ROST, B. 2007. ISIS: interaction sites identified from sequence. In *Bioinformatics,* vol. *23*, pp. e13-e16. DOI: 10.1093/bioinformatics/btl303

PATEL, S.M. – KORINGA, P.G. – REDDY, B.B. – NATHANI, N.M. – JOSHI, C.G. 2015. In silico analysis of consequences of non-synonymous SNPs of Slc11a2 gene in Indian bovines. In *Genomics Data*, vol. *5*, pp. 72–79. DOI: 10.1016/j.gdata.2015.05.015

PALINE, C.N. – STEVEN, H. 2003. SIFT: predicting amino acid changes that affect protein function. In *Nucleic Acids Research,* vol. *31*, pp. 3812–73814. DOI: 10.1093/nar/gkg509

RAFALSKI, A. 2002. Applications of single nucleotide polymorphisms in crop genetics. In *Current Opinion in Plant Biology,* vol. *5*, pp. 94–100. DOI: 10.1016/S1369-5266(02)00240-6

ROMERO, P.R. – ZAIDI, S. – FANG, Y.Y. – UVERSKY, V.N. – RADIVOJAC, P. – OLDFIELD, C.J. – CORTESE, M.S. – LEGALL, T. – OBRADOVIC, Z. – DUNKER, A.K. 2006. Alternative splicing in concert with protein intrinsic disorder enables increased functional diversity in multicellular organisms. In *Proceedings of the National Academy of Sciences USA*, vol. *103*, pp. 8390–8395. DOI: 10.1073/pnas.0507916103

SALDANHA, R. – CHEN, B. – WANK, H. – MATSUURA, M. – EDWARDS, J. – LAMBOWITZ, A.M. 1999. RNA and protein catalysis in group II intron splicing and mobility reactions using purified components. In *Biochemistry*, vol. *38*, pp. 9069–9083. DOI: 10.1021/bi982799l

SAUNA, Z.E. – KIMCHI-SARFATY, C. 2011. Understanding the contribution of synonymous mutations to human disease. In *Nature Review Genetics,* vol. *12*, pp. 638–691. DOI: 10.1038/nrg3051

SCHLESSINGER, A. – PUNT, A.M. – YACHDAV, G. – KAJAN, L. – ROST, B. 2009. Improved disorder prediction by combination of orthogonal approaches. In *PLoS One*, vol. *4*, no. 2, pp. e4433. DOI: http://dx.doi.org/10.1371/journal.pone.0004433

SCHNEIDER, K. – WEISSHAAR, B. – BORCHARDT, D.C. – SALAMINI, F. 2001. SNP frequency and allelic haplotype structure of Beta vulgaris expressed genes. In *Molecular Breeding*, vol. *8*, pp. 63–74. DOI: 10.1023/A:1011902916194

SCHOLTZ, S. – LORZ, H. – LUTTICKE, S. 2001. Transposition of maize transposable element Ac in barley (*Hordeum vulgare* L.). In *Molecular and General Genetics*, vol. *264*, pp. 653–661. DOI: 10.1007/s004380000351

SMIGIELSKI, E.M. – SIROTKIN, K. – WARD, M. – SHERRY, S.T. 2000. dbSNP: a database of single nucleotide polymorphisms. In *Nucleic Acids Research,* vol. *28*, no. 1, pp. 52–355. url: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC102496/pdf/gkd114.pdf

VON BOTHMER, R. – SATO, K. – KOMATSUDAM, T. – YASUDA, S. – FISCHBECK, G. 2003. The domestication of cultivated barley. In von BOTHMER, R. – VAN HINTUM, T. – KNÜPFFER, H. – SATO, K. (Eds.) *Diversity in barley* (*Hordeum vulgare*). Amsterdam: Elsevier Science BV, pp. 9–27.

WARD, J.J. – SODHI, J.S. – MCGUFFIN, L.J. – BUXTON, B.F. – JONES, D.T. 2004. Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. In *Journal of Molecular Biology*, vol. *337*, pp. 635–645. DOI: 10.1016/j.jmb.2004.02.002

YU, J. – XUE, J.H. – ZHOU, S.L. 2011. New universal *mat*K primers for DNA barcoding angiosperms. In *Journal of Systematic Evolution*, vol. *49*, no. 3, pp. 176. DOI: 10.1111/j.1759-6831.2011.00134.x

ZOSCHKE, R. – NAKAMURA, M. – LIERE, K. – SUGIURA, M. – BORNER, T. – SCHMITZ-LINNEWEBER, C. 2010. An organellar maturase associates with multiple group II introns. In *Proceedings of the National Academy of Sciences USA*, vol. *107*, no. 7, pp. 3245. DOI: 10.1073/pnas.0909400107

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APPLICATION OF VEGETATIVE BUFFER STRIPS UNDER NATURAL RAINFALL TO CONSERVE SOIL AND WATER

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Soil erosion is one of the most serious environmental issues in the world. The use of vegetative buffer strips is an effective strategy to reduce surface water pollutions as well as soil erosion. The present research has been conducted with aim to study the efficiency of buffer strips in runoff volume and sediment control using experimental plots. In this regard, twelve experimental plots with the dimensions of 1 m × 10 m were provided, and runoff samples were collected monthly to measure runoff volume and sediment. Vetiver grass and tall fescue were used as the plant species of the studied vegetative buffer strips. It was found that, vegetative buffer strips reduced the runoff volume up to 97% and sediment concentration up to 96%. Vetiver grass showed a high efficiency in runoff and sediment control; but, the maximum efficiency is achieved when this species is used along with a plant similar to the tall fescue in terms of density and uniformity. Also, periodic cutting the plants and cleaning the buffer strips can be considered as effective strategies to prevent vegetative buffer strips acting as the source of sediment.

Key words: sediment, vetiver grass, tall fescue, runoff volume, Sari

Soil erosion is one of the most serious environmental issues around the world. This problem occurs by soil exposure due to loss of vegetation cover which causes soil and water capacity reduction, pollution and eutrophication of water bodies (Morgan 1995; Hay *et al*. 2006; Keesstra *et al*. 2016; Kavian *et al.* 2017; Rodrigo-Comino *et al.* 2018). The use of vegetative buffer strips is a measure to deal with the mentioned issues (Yuan *et al*. 2009). Vegetative buffer strips include various plants such as grass, tree and shrub installed at the downstream of erodible and agricultural lands as well as river banks (Dabney 2003; Saleh *et al.* 2018). The mentioned strips are generally used for surface flows, sediment trapping, nutrient filtering and providing appropriate aquatic habitat (Yuan *et al*. 2009).

Vetiver grass (*Vetivera zizanioides*). Vetiver grass is widely used as a bioengineering technique to stabilize slopes, phytoremediation of polluted land and water, and many other environmental conservation measures (Shooshtarian & Tehranifar 2011). This plant is a fast-growing species with a height of 50–150 cm and an extent of 30 cm. The roots of vetiver grass are so branched and bulky those which penetrate up to the depths of 2–4 m in the soil; so, it is very effective for soil and water conservation (Iranian Association for Vetiver Promotion 2008). Vetiver grass is compatible with different climatic conditions, such as flooding, long-term drought, and temperatures in the range of 14 to 55ºC. Also, this plant is capable to re-grow after environmental stresses such as salinity, drought

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and etc. Tolerance of a wide range of soil acidity is another characteristic of vetiver grass as well as resistance to the herbicides and pesticides.

Tall fescue *(Festuca arundinacea).* This plant species is able to increase the soil permeability and create the sheet flow due to the proper density and fast growth. So, the tall fescue can be considered as a suitable plant to be used in vegetative buffer strips.

Many studies have been conducted on the effect of vegetative buffer strips on runoff quality and quantity control (Norris 1993; Delgado *et al.* 1995; Lee *et al*. 2003; Patty *et al*. 1997; Golabi *et al*. 2005; Borina *et al*. 2005; Hay *et al*. 2006; Mankin *et al*. 2007; Duchemin & Hogue 2009; Borin *et al*. 2010; Milan *et al*. 2014). Some researchers have attempted to give a guideline for using the vegetative buffer strips for the water quality control. They believed that, the proximity of vegetative buffer strips to the source of contaminations may play an important role in their efficiency (Norris 1993). Hay *et al*. (2006) conducted an experimental study to evaluation of the impacts of the vegetative buffer strips on removing some pollutants generated by irrigated lands and rangelands, filter strips may not have high efficiency, because of high runoff volume, high slope and channelized flow. Investigation of hydraulic characteristics of runoff and sediment production in steep plots covered by grass has shown that, the plot covered by grass has less runoff and sediment by 14–25% and 81–95% respectively, than the plot control (Pan & Shangguan 2006). Lambrechts *et al*. 2014 studied the effect of plant and its morphology on the efficiency of vegetative buffer strips using experimental flume. They indicated the high sediment trapping potential of vegetative buffer strips after two months growing. According to the reports of the researcher above, plant growth increases sediment trapping by the vegetative buffer strips. Patty *et al.* (1997) conducted a study and stated that the grass strips with lengths of 6, 12 and 18 m were reduced the runoff volume by 87–100% and suspended solids by 44–100%. Lee *et al.* (2003) believed that a combination of various plants can enhance the effectiveness of the vegetative buffer strips for runoff pollution removal. Evaluation of the effect of a grass-tree system on filtering the runoff generated by a corn field fertilised by manure indicated that a grass strip can remove the runoff volume by 40%, suspended solids by 87%, total phosphorous by 64% and nitrate up to about 33%; while, the grass-tree strips reduced the runoff volume by 35%, suspended solids by 85%, total phosphorous by 85% and nitrate up to about 30% (Duchemin & Hogue 2009). After reviewing the data obtained from the studies on the performance of vegetative buffer strips in Italy, Borin *et al.* (2010) reported that the young buffer strips can reduce the phosphorous loss up to about 50%, nitrogen loss up to 44% and runoff volume by 33% as compared to the bare areas. Wakida *et al.* (2014) found a high correlation between the concentrations of suspended solids, phosphorus, chemical oxygen demand and turbidity, but not for total nitrogen in the Tijuana city. Campo-Bescos *et al.* (2015) believed that the installation of intense vegetative buffers in irrigated lands can improve the environmental conservation. However, it should not be considered as a main strategy; but also, it should be used as a supplementary pollution control approach along with other measures outside the field.

As many studies showed, the effectiveness of the vegetative buffer strips in runoff reduction and sediment removal; however, the impact of plant species on the efficiency of buffer strips has been less studied on plot scale.

The present study evaluated the impact of vetiver grass (*Vetivera zizanioides*) and native tall fescue (*Festuca arundinacea*) in runoff volume reduction and sediment removal using experimental plots of Sari (Iran) and the combination of these species on the efficiency of vegetative buffer strips in runoff volume reduction and sediment removal using experimental plots.

MATERIAL AND METHODS

Site description

The study site includes a part of rain-fed croplands of Miandorood (Mazandaran, Iran) where the wheat is grown. The study area is located at the eastern longitude of 53º10′ and northern latitude of 36º 33′ at the northern hemisphere (Figure 1).

Table 1 shows some characteristics of the studied site based on Dasht-e-Naz station weather data (Sadeghi Ravesh 2011).

Table 1

Characteristics of the studied site

Land use	Soil texture classification (USDA)	Soil classification (USCS)	Soil type	Elevation from sea level [m]	Slope [%]	Mean annual temperature [ºC]	Relative humidity [%]
Cropland (wheat)	Clay-loam	OH	Non-saline	23	15	17	77

Experimental design

The present study includes a one-year experiment. Twelve experimental plots with the dimensions of 1 m × 10 m and the slope of 15% were provided as randomized complete block design; so that, there were four treatments including vetiver grass, native tall fescue, combination of vetiver grass and native tall fescue, and bare (control) plots with three replications.

In the present study, experimental plots used those which were isolated with the intervals of 10 cm deep in the soil using galvanized sheets (Lee *et al.* 1999; Kelarestaghi *et al.* 2008) (Figure 2). Also, a path was created at the downslope of each plot to drain the outflow into a 120 L tank. The studied plants cultivated in late January and divided into two parts with the lengths of 3 m and 7 m. In 3 m part, the studied plant species were cultivated and the remaining 7 m was left as bare. The vegetation cover of the studied plants also was monitored during the experiment period (Table 2).

Runoff sampling

The runoff samples were taken from the rainfall water collected by tanks existing at downslope of each plot monthly since February 2015 until Jan-

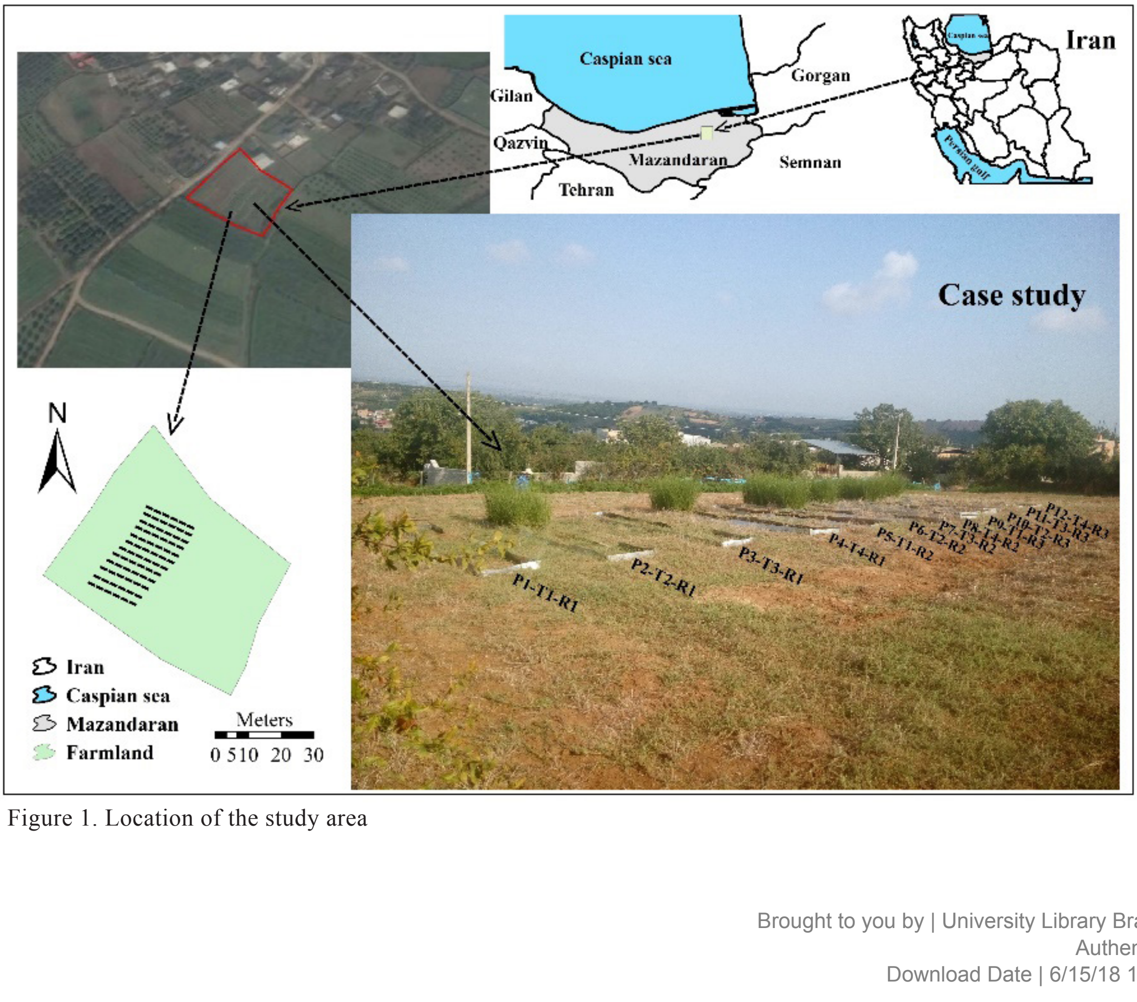

Figure 1. Location of the study area

Table 2

The vegetation cover of the studied plants during the experiment period

Plant species	Feb 2015	Mar 2015	Apr 2015	May 2015	Jun 2015	Jul 2015	Aug 2015	Sep 2015	Oct 2015	Nov 2015	Dec 2015	Jan 2016
			Vegetation cover [%]									
Tall fescue	60	65	65	60	40	30	30	30	25	25	25	25
Vetiver grass	30	65	90	90	90	90	90	90	90	90	90	90

uary 2016. Before measuring the outflow volume, a 1.5 L sample was taken in order to determine sediment concentration (Lee *et al*. 1999; Kavian *et al.* 2014). Also, the amount of precipitation was measured during the experiment period using a storage rain-gauge (Table 3).

Pollutants measurement

Nitrate and phosphate concentrations were measured in the laboratory of Regional Water Office of Sari (Mazandaran, Iran). In order to measure the sediment concentration of the water samples, the samples were firstly weighted and then, were dried under temperature of 105°C in an oven for 24 hours. Finally, the samples were weighted again to obtain the weight of dry sediment. Equation (1) calculates the amount of total suspended solids of water samples (Lee *et al.* 1999; Mohammadi & Kavian 2015).

$$\mathrm{TSS} = \frac{\mathrm{M}}{\mathrm{V}} \qquad \text{Equation (1)}$$

where: M is the weight of dry solids [mg] and V is the volume of water sample [L].

Determination of the efficiency of the vegetative buffer strips

Efficiency of the studied vegetative buffer strips in runoff volume reduction and sediment removal calculated using Equation (2) (Lee *et al.* 1999).

$$\text{Effectiveness } (\mathrm{T_1}) = (1 - \frac{\mathrm{P_1}}{\mathrm{P_1}}) \times 100 \qquad \text{Equation (2)}$$

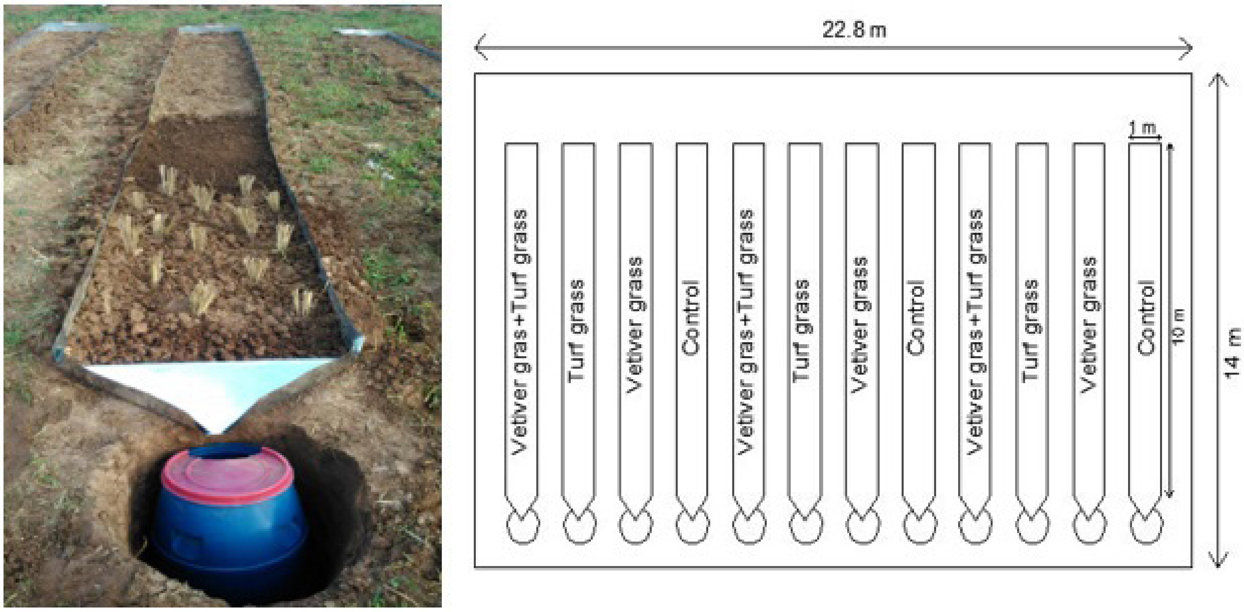

Figure 2. Installation of the experimental plots

where: T_i is the efficiency of treatment (i) [%], P_i is the value of sediment concentration (runoff volume) in the runoff sample of the treatment (i) and P_1 is the value of sediment concentration (runoff volume) in the runoff sample of the control plot.

Statistical analysis

First, a data base was provided in Excel software (2013) and then, the normality test for the data was carried out using Kolmogorov-Smirnov approach. Finally, comparison of means was conducted using SPSS software Version 18 to compare the performance of different treatments sediment removal and runoff volume reduction (SPSS Ink 2009).

RESULTS

Runoff volume

According to the results the treatment of tall fescue has the minimum amount of runoff volume flowing out of the experimental plots in the first and second months of the experiment with a significant difference (*P* = 0.01) compared to the other studied treatments. It is due to the fast growth of tall fescue and higher density compared to the vetiver grass during the mentioned period. The treatment of vetiver grass-tall fescue showed the least outflow volume since the fourth month until the 10th month. This performance is due to the growth of vetiver grass and higher soil permeability caused by the vetiver roots in the mentioned period. After the 10th month when the density of tall fescue was reduced due to climatic conditions and grazing, the treatment of vetiver grass-tall fescue had the minimum amount of runoff volume along with the treatment of the vetiver grass (Figure 3).

Figure 4 represents the best performance of runoff volume reduction for the treatment of tall fescue in the first (66%) and second (76%) months. The treatment of vetiver grass-tall fescue showed the highest efficiency compared to the other treatments

Table 3

The amount of precipitation during the experiment period

Feb 2015	Mar 2015	Apr 2015	May 2015	Jun 2015	Jul 2015	Aug 2015	Sep 2015	Oct 2015	Nov 2015	Dec 2015	Jan 2016
Amount of precipitation [mm]											
19.7	23	118.8	15	5.4	3.5	83.8	3.6	112.6	112.5	100.4	55.3

Table 4

Comparison of the means of sediment concentration in the four studied treatments during the experiments

Treatment	Feb 2015	Mar 2015	Apr 2015	May 2015	Jun 2015	Jul 2015	Aug 2015	Sep 2015	Oct 2015	Nov 2015	Dec 2015	Jan 2016
	Sediment concentration [g/l]*											
Control	56.35^{b}	53.61^{c}	42.51^{c}	60.55^{d}	86.29^{c}	97.30^{c}	54.13^{d}	95.00^{d}	44.83^{c}	49.07^{d}	53.63^{d}	56.97^{c}
Vetiver grass	27.61^{a}	17.69^{b}	6.38^{b}	7.27^{a}	13.81^{b}	15.57^{a}	9.74^{a}	14.25^{a}	9.41^{a}	9.81^{a}	13.41^{a}	15.38^{a}
Tall fescue	20.29^{a}	5.90^{a}	5.10^{ab}	9.69^{c}	18.12^{b}	27.24^{b}	21.65^{c}	37.05^{c}	21.97^{b}	27.48^{c}	30.03^{c}	34.18^{b}
Vetiver grass-tall fescue	25.36^{a}	13.94^{b}	2.13^{a}	2.42^{b}	4.31^{a}	22.38^{ab}	15.70^{b}	25.65^{b}	17.04^{b}	16.68^{b}	21.45^{b}	23.93^{b}

*Means followed by the same letter do not differ statistically

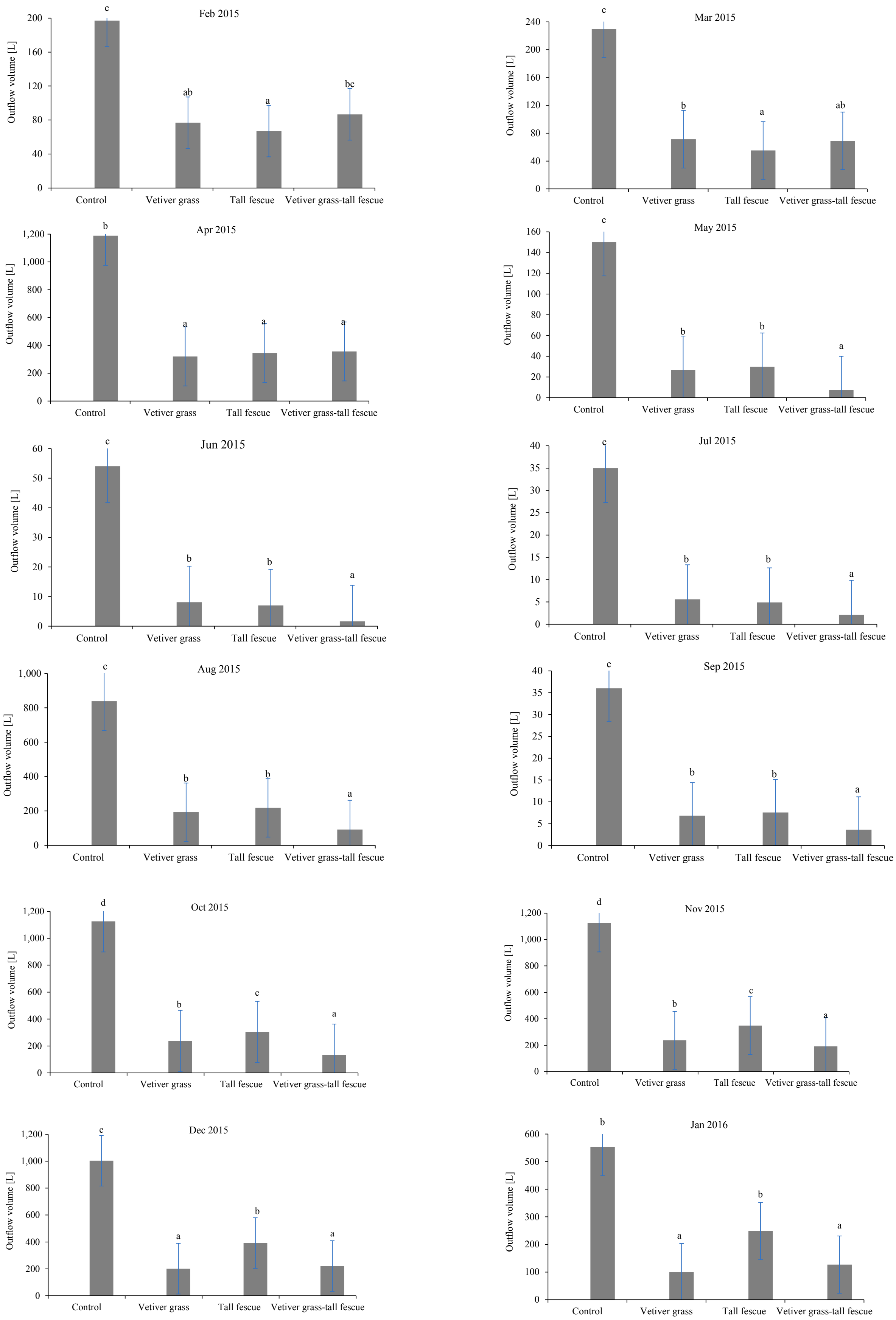

Figure 3. Variations of mean outflow volume in the four studied treatments during the experiment (Means followed by the same letter do not differ statistically)

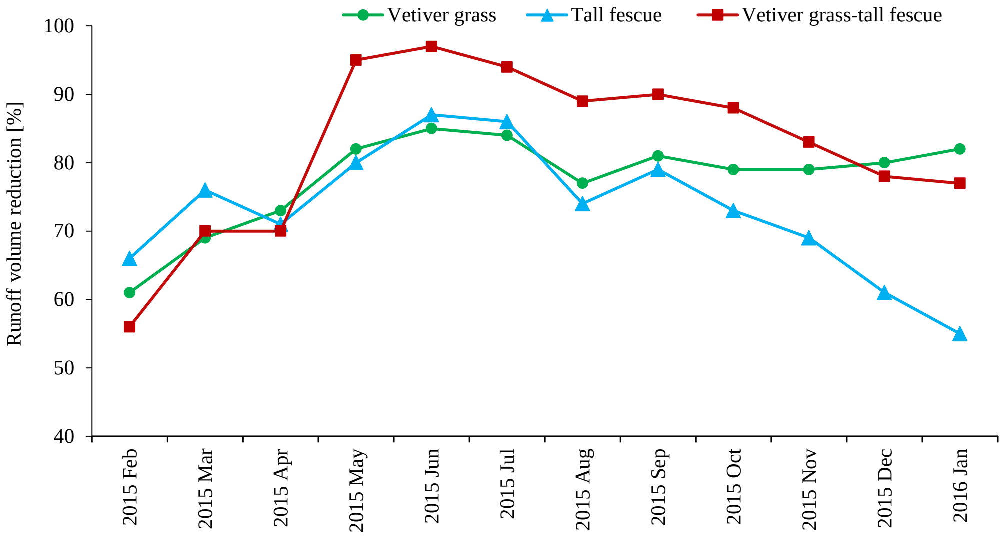

Figure 4. Variations of the efficiency of the studied vegetative buffer strips in runoff volume reduction during the experiment

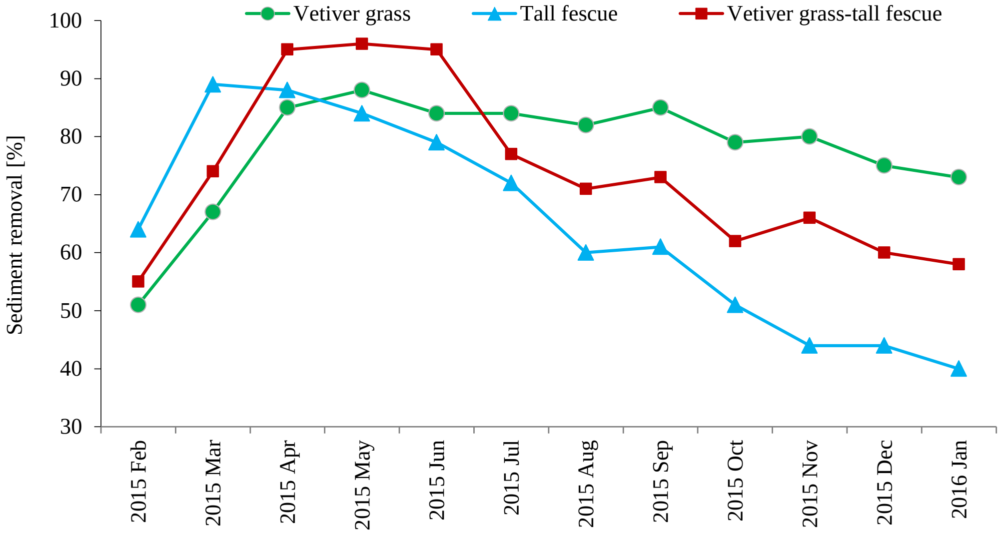

Figure 5. Variations of the efficiency of the studied vegetative buffer strips in sediment removal during the experiment

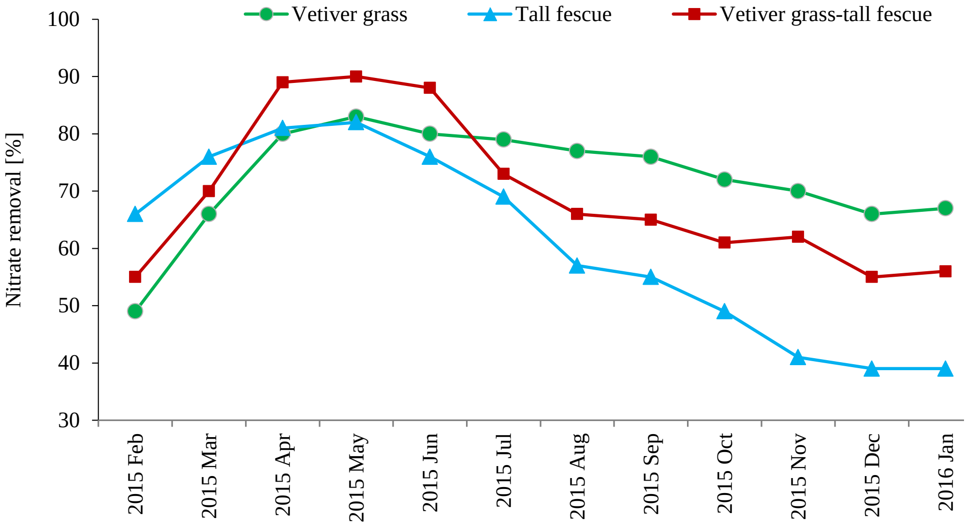

Figure 6. Variations of the efficiency of the studied vegetative buffer strips in nitrate removal during the experiment

since the fourth month until the 10th month of the experiment. Moreover, the best efficiency was obtained in the 11th and 12th months for the treatment of vetiver grass. As it is observed, the highest efficiency (97%) was found for the treatment of vetiver grass-tall fescue in the fifth month.

Sediment concentration

After some fluctuations, the treatment of vetiver grass showed the minimum amount of sediment concentration with a significant difference ($P = 0.01$) compared to the other treatments since the sixth month until the end of the experiment (Table 4). The main reasons include the ability of vetiver grass for sediment removal and its compatibility with different climatic conditions, as well as the vulnerability of tall fescue.

As Figure 5 shows, the maximum efficiency in the first and second months was observed for the treatment of tall fescue; while the treatment of vetiver grass-tall fescue had more appropriate performance than the other treatments in the third, fourth and fifth months. Since the sixth month of the experiment, the treatment of vetiver grass showed the best efficiency of sediment removal until the end of the experiment. The highest efficiency (96%) during the experiment period was also related to the treatment of vetiver grass-tall fescue in the fourth month.

Nitrate

According to Figure 6, the maximum efficiency (90%) in nitrate removal was found in the fourth month of the experiment for the treatment of vetiver grass-tall fescue. But, the treatment of vetiver grass showed the best performance since the sixth month until the end of the experiment.

Phosphate

As the results indicate, the best function of phosphate removal was determined in the third month by the treatment of vetiver grass-tall fescue. Since the sixth month of the experiment, the highest efficiency was obtained by the treatment of vetiver grass until the end of the experiment (Figure 7).

DISCUSSION

According to the results (Figure 4), the treatment of tall fescue has had the maximum runoff reduction in the first and second months of the experiment which is consistent with Owino *et al.* (2006) and Yuan *et al.* (2009). Since the third month when the tall fescue and vetiver grass had their maximum vegetation cover, the treatment of vetiver grass-tall fescue showed the minimum outflow volume. The maximum efficiency of the buffer strips in qualitative

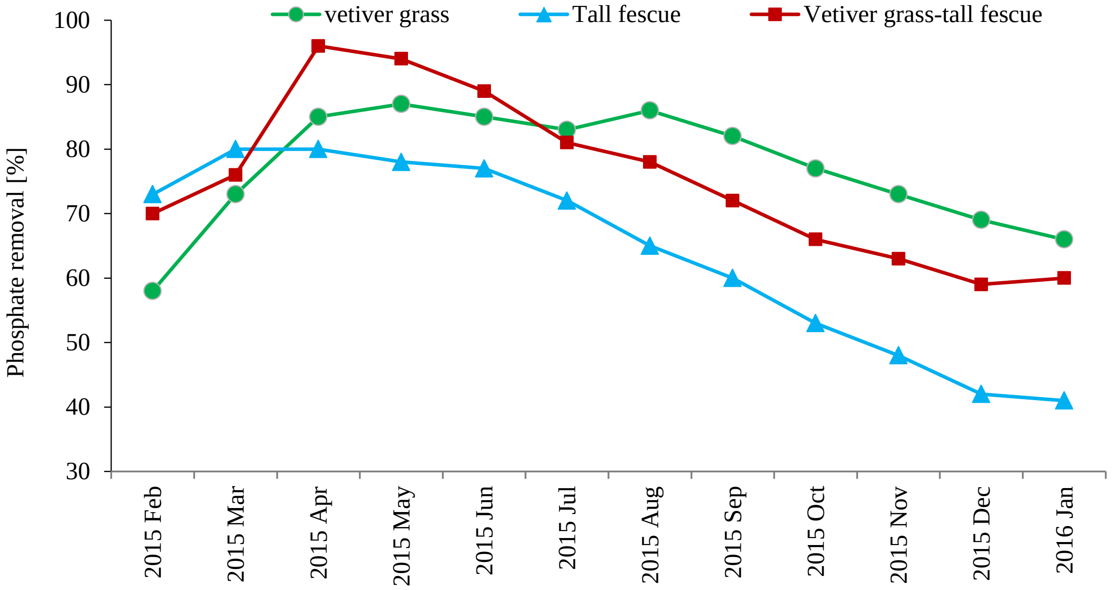

Figure 7. Variations of the efficiency of the studied vegetative buffer strips in phosphate removal during the experiment

and quantitative control of the runoff occurs when the flow passes through the strips as a sheet (Hussein *et al*. 2007). So, the highest efficiency in the runoff volume reduction was found for the treatment of vetiver grass-tall fescue because the runoff reached to the vetiver grass strip as a sheet flow after passing through the tall fescue strip. Hence, the flow was not concentrated among the bushes of vetiver grass; so, the strip of vetiver grass could present its capability to increase soil permeability. Lee *et al*. (2003) also obtained the same result which explains the proper performance of the combined vegetative buffer strips to control runoff volume and pollutants. In the last two months of the experiment, the vegetation cover of tall fescue was reduced because of changing the climatic conditions as well as grazing over the time. Therefore, the efficiency of tall fescue strip dropped while the vegetative strip kept its high efficiency in runoff volume reduction because of strong root system, higher biomass as well as compatibility to various climatic conditions. This result is consistent with Mankin *et al*. (2007) and Golabi *et al*. (2005).

Investigating the results of sediment concentration (Figure 5) represented that, the combination of vetiver grass and tall fescue can give a high efficiency in sediment removal. But, when the vegetation cover of tall fescue reduced (since the sixth month), the treatment of vetiver grass showed the highest efficiency of sediment removal compared to the other treatments until the end of the experiment. The high performance of vetiver grass is due to high resistance in various seasons and climatic conditions as well as dormancy in cold season. So, vegetation cover reduction is prevented. The achieved results are consistent with Golabi *et al*. (2005), Pan & Shanggun (2006) and Stutter *et al*. (2009).

By approaching the end of experiment, the mean concentration of sediment was higher than the early months of the experiment. This happening can be related to the sediment accumulation in the vegetative strips over time. Therefore, the vegetative buffer strips can play role as the source of sediment. Osborne & Kovacic (1993), Bhattarai *et al*. (2009) and Stutter *et al*. (2009) also obtained the same result.

The results of nitrate and phosphate (Figure 6 and 7) also represent proper function for vetiver grass-tall fescue when the mentioned plants have their maximum growth and vegetation cover. While, the treatment of vetiver grass showed higher stability than the composed buffer strip for nitrate removal due to higher resistance against grazing and climatic condition changes. This result is consistent with Matteo *et al.* (2006) and Lee *et al.* (2003). The achieved results for nitrate and phosphate are almost same as the sediment; because, the nutrients are attached to the sediment particles and transferred (Barling 1994).

CONCLUSIONS

The plots with vetiver and tall fescue buffer strips both were able to reduce runoff, sediment, nitrate and phosphate concentrations as compared to the control. But, the vetiver grass was more effective than other treatments when the plants reached maturity. Also, tall fescue showed less effective than vetiver grass at reducing in runoff, pollutant transport and soil erosion. Because, the effectiveness of tall fescue sharply decreased after the second month since planting. Therefore, the appropriate effectiveness of vetiver grass-tall fescue treatment reduced after a short time. Hence, a more appropriate efficiency for water and soil conservation will be achieved if a plant species with a density and uniformity like the tall fescue being resistant and compatible to the climatic conditions of the considered region is used along with vetiver grass in the vegetative buffer strips. The results of this study showed that the vegetative buffer strips can also act as a source of nutrients and sediment. So, periodic plants cutting and dredging of buffer strips is recommended as an effective strategy to deal with this problem. In addition, doing researches on the width of the strips, the impact of length and shape of the sub/catchment above the strip, rainfall intensity and soil moisture on the efficiency of the vegetative buffer strips can be helpful to enhance the impact of these strips in the water and soil conservation in different watersheds.

REFERENCES

BARLING, R.D. 1994. Role of Buffer Strips in Management of Waterway Pollution: A Review. In *Environmental Management,* vol. *18*, vol. *4,* pp. 543–558.

BHATTARAI, R. – KALITA, P.K. – PATEL, M.K. 2009. Nutrient transport through vegetative filter strip with subsurface drainage. In *Journal of Environmental Management*, vol. *90*, pp. 1868–1876.

BORIN, M. – PASSONI, M. – THIENE, M. – TEMPESTA, T. 2010. Multiple functions of buffer strips in farming areas. In *European Journal of Agronomy*, vol. *32*, pp. 103–111.

BORINA, M. – VIANELLOA, M. – MORARIA, F. – ZANIN-BG. 2005. Effectiveness of buffer strips in removing pollutants in runoff from a cultivated field in North-East Italy. In *Agriculture, Ecosystems and Environment*, vol. *105*, pp. 101–114.

CAMPO-BESCOS, M.A. – MUNOZ-CARPENA, R. – KIKER, G.A. – BODAH, B.W. – ULLMAN, J.L. 2015. Watering or buffering? Runoff and sediment pollution control from furrow irrigated fields in arid environments. In *Agriculture, Ecosystems and Environment*, vol. *205*, pp. 90–101.

DABNEY, S.M. 2003. *Erosion control, vegetative*. In *Encyclopaedia of Water Science*. New York, USA: Marcel Dekker, Madison Ave, pp. 272–276.

DELGADO, A.N. – PERIAGO, E.L. – VIQUERIA, F.D. 1995. Vegetated filter strips for wastewater purification: a review. In *Bioresource Technology*, vol. *51*, pp. 13–22.

DUCHEMIN, M. – HOGUE, R. 2009. Reduction in agricultural non-point source pollution in the first year following establishment of an integrated grass/tree filter strip system in southern Quebec (Canada). In *Agriculture, Ecosystems and Environment*, vol. *131*, pp. 85–97.

GOLABI, M.H. – IYEKAR, C. – MINTON, D. – RAULERSON, C.L. – DRAKE, J.C. 2005. Watershed management to meet water quality standards by using the vetiver system in Southern Guam. In *Austral Journal of Technology,* vol. *9*, pp. 63–70.

HAY, V. – PITTROFF, W. – TOOMAN, E.E. – MEYER, D. 2006. Effectiveness of vegetative filter strips in attenuating nutrient and sediment runoff from irrigated pastures. In *Journal of Agricultural Science*, vol. *144*, pp. 349–360.

HUSSEIN, J. – YU, B. – GHADIRI, H. – ROSE, C. 2007. Prediction of surface flow hydrology and sediment retention upslope of a vetiver buffer strip. In *Journal of Hydrology*, vol. *338*, pp. 261– 272.

IRANIAN ASSOCIATION FOR VETIVER PROMOTUION. 2011. *Vetiver system to prevent and treatment of chemical pollutions of water and soil.* 1st National Phytoremediation Conference, Kerman, Iran, 580 p. (In Persian)

KAVIAN, A. – AZMOODEH, A. – SOLAIMANI, K. 2014. Deforestation effects on soil properties, runoff and erosion in northern Iran. In *Arabian Journal of Geosciences*, vol. *7*, no. 5, pp. 1941–1950.

KAVIAN, A. – HOSEINPOUR SABET, S. – SOLAIMANI, K. – JAFARI, B. 2017. Simulating the effects of land use changes on soil erosion using RUSLE model. In *Geocarto International*, vol. *32*, no. 1, pp. 97–111.

KEESSTRA, S. – PEREIRA, P. – NOVARA, A. – BREVIK, E.C. – AZORIN-MOLINA, C. – PARRAS-ALCÁNTARA, L. – JORDÁN, A. – CERDÀ, A. 2016. Effects of soil management techniques on soil water erosion in apricot orchards. In *Science of the Total Environment,* vol. *551–552*, pp. 357–366.

KELARESTAGHI, A.A. – AHMADI, H. – ESMAEILI ORI, A. – GHODUSI, J. 2008. Comparison of runoff and sediment production in various agricultural land use treatments. In *Iran-Watershed Management Science and Engineering*, vol. *2*, no. 5, pp. 41–52.

LAMBRETCHS, T. – FRANCOIS, S. – LUTTS, S. – MUNOZ-CARPENA, R. – BIELDERS, C. 2014. Impact of plant growth and morphology and of sediment concentration on sediment retention efficiency of vegetative filter strips: Flume experiments and VFSMOD modeling. In *Journal of Hydrology,* vol. *511*, pp. 800–810.

LEE, K.H. – ISENHART, T.M. – SCHULTZ, R.C. – MICKELSON, K.S. 1999. Nutrient and sediment removal by switchgrass and cool-season filter strips in Central Iowa, USA. In *Agroforestry Systems*, vol. *44*, pp. 121–132.

LEE, K.H. – ISENHART, T.M. – SCHULTZ, R.C. 2003. Sediment and nutrient removal in an established multi-species riparian buffer. In *Journal of Soil and Water Conservation*, vol. *58*, no. 1, pp. 1–8.

MANKIN, K.R. – DANIEL, M.N. – CHARLES, J.B. – STACY, L.H. – WAYNE, A.G. 2007. Grass-shrub riparian buffer removal of sediment, phosphorus, and nitrogen from simulated runoff. In *Journal of the American Water Resources Association* (JAWRA), vol. *43*, no. 5, pp. 1108–1116.

MATTEO, M. – RANDHIR, T. – BLONIARZ, D. 2006. Watershed-scale impacts of forest buffers on water quality and runoff in urbanizing environment. In *Journal of Water Resources Planning and Management*, vol. *132*, no. 3, pp. 144–152.

MILAN, M. – FERRERO, A. – LETEY, M. – DE PALO, F. – VIDOTTO, F. 2014. Effect of buffer strips and soil texture on runoff losses of flufenacet and isoxaflutole from maize fields. In *Journal of Environmental Science and Health*, Part B, vol. *48*, pp. 1021–1033.

MOHAMMADI, M.A. – KAVIAN, A. 2015. Effects of rainfall patterns on runoff and soil erosion in field plots. In *International Soil and Water Conservation Research,* vol. *3*, no. 4, pp. 273–281.

MORGAN, R.P.C. 1995. *Soil erosion and conservation*, 2nd edition. Longman, Addison, pp. 175–176.

NORRIS, V. 1993. The use of buffer zones to protect water quality: A review. In *Water Resources Management*, vol. *7*, pp. 257–272.

OSBORNE, L.L. – KOVACIC, D.A. 1993. Riparian vegetated buffer strips in water-quality restoration and stream management. In *Freshmler Biology,* vol. *29*, pp. 243–258.

OWINO, J.O. – OWIDO, S.F.O. – CHEMELIL, M.C. 2006. Nutrients in runoff from a clay loam soil protected by narrow grass strips. In *Soil & Tillage Research*, vol. *88*, pp. 116–122.

PAN, CH. – SHANGGUN, Z. 2006. Runoff hydraulic characteristics and sediment generation in sloped grassplots under simulated rainfall conditions. In *Journal of Hydrology*, vol. *331*, pp. 178–185.

PATTY, L. – REAL, B. – GRIL, J. 1997. The use of grassed buffer strips to remove pesticides, nitrate and soluble phosphorus compounds from runoff water. In *Pest Management Science*, vol. *49*, pp. 243–251.

RODROGO-COMINO, J. – DAVIS, J. – KEESSTRA, S.D. – CERDA, A. 2018. Updated measurements in vineyards improves accuracy of soil erosion rates. In *Agronomy Journal,* vol. *110*, no. 1, pp. 411–417.

SADEGHI RAVESH, M.H. 2011. Comparison of human thermal comfort amount in arid and humid climates (Case study: Yazd and Sari cities). In *Arid Biom Scientific and Research Journal*, vol. *1*, no. 2, pp. 50–61.

SALEH, I. – KAVIAN, A. – HABIBNEJAD, M. – JAFARIAN, Z. 2017. The efficiency of vegetative buffer strips in runoff quality and quantity control. In *International Journal of Environmental Science and Technology,* pp. 1–10. DOI: 10.1007/s13762-017-1411-2. Article in Press.

SOOSHTARIAN, S. – TEHRANIFAR, A. 2011. *Introducing the vetiver grass*. Iranian Agricultural Science Publisher, 170 p.

SPSS INK. 2009. PASW Statistics for OSX, Version 18.0. SPSS Inc., Chicago.

STUTTER, M. – LANGAN, S. – LUMSDON, A. 2009. Vegetated buffer strips can lead to increased release of phosphorus to waters: A biogeochemical assessment of the mechanisms. In *Environment Science Technology*, vol. *43*, pp. 1858–1863.

WAKIDA, F.T. – MARTINEZ-HUATO, S. – GARCIA-FLORES, E. – PINON-COLIN, T.D.G. ESPINOZA-GOMEZ, H. – AMES-LOPEZ, A. 2014. Pollutant association with suspended solids in stormwater in Tijuana, Mexico. In *International Journal of Environmental Science and Technology*, vol. *11*, no. 2, pp. 319–326.

YUAN, Y. – BINGNER, R.L. – LOCKE, M.A. 2009. A Review of effectiveness of vegetative buffers on sediment trapping in agricultural areas. In *Journal of Ecohydrology*, vol. *2*, pp. 321–336.

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Original paper

THE EFFECT OF DIFFERENT TILLAGE METHODS ON EROSION

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During the years 2012–2016 at the site threatened by erosion, the effect of different intensity and depth of soil tillage on the progression of erosion were evaluated on the plots with silage maize. Three different tillage methods were compared and evaluated – conventional tillage, including ploughing (CT), no-tillage using mulch and direct drilling (NT), and minimum tillage treatment with a lower depth of soil cultivation and organic matter incorporation (MT). Water and soil runoff on all of the experimental plots were measured during erosion events. Besides an analysis of naturally occurring rainfall causing erosions, we also conducted the test of soil infiltration abilities with a rain simulator after silage maize harvest. The effect of the tillage on aboveground biomass yield and the input costs was also analyzed. The results showed that NT and MT can significantly reduce water and soil runoff comparing CT. The highest yields were recorded in MT, while the lowest were in CT. Total input costs were higher in the case of NT and MT, but the share of mechanized work was lower for these technologies. Our results showed that NT and MT technologies, as a part of silage maize with a higher plant density stand establishment, should be a useable erosion control measure in areas vulnerable to erosion.

Key words: Cambisol, conventional tillage, no tillage, minimum tillage, water erosion, soil and water runoff, grain yield

Erosion is a worldwide phenomenon that harms thousands of square kilometers of arable land. Billions of tons of topsoil are removed every year (Morgan 2005). Keller *et al*. 2013 published that in the United Kingdom about 40% of arable land is devastated each year by water erosion. It is estimated that around 3 million tonnes of high-quality soil are transported every year to drainage systems and rivers. Approximately 54% of arable land is menaced by water erosion in the Czech Republic nowadays.

According to experts, the compensations of soil losses larger than 2 t/ha a year will last approximately 50 or 100 years (Holý 1994). No universal solutions, to deal with erosion, exist. But there are several options of how to deal with erosion or to decrease erosion effects. Globally, the issue of soil erosion is solved primarily by optimizing the size and shape of the fields (by dividing fields into strips), by optimizing crop rotations, by using soil protection technologies via reduction of the intensity of soil cultivation, and decreases of working operations (Martens *et al*. 2000; Sainju 2002; Hernanz *et al.* 2002), or by keeping the residues of the preceding crops at the field. Using the conservation tillage can significantly decrease negative kinetic effect of rain drops during the torrential rains (Trauman *et al*. 2005; Kovaříček *et al*. 2008) and increase the microbial community and activity, doubling the resistance against erosion (Bhatt & Khera 2006; Mikanová *et al.* 2009; Šimon *et al.* 2009). Conservation tillage not only reduces erosion (Basić *et al*. 2004; Schuller *et al*. 2007), but also positively influences the soil's ability of retention, its structure, fertility, and other parameters (Tebrügge & Düring 1999; Zhang *et al*. 2007).

The aim of our work was to analyze optimal tillage systems for silage maize, which would minimize water erosion and soil losses and compare these systems to how they influence grain yields of silage maize and input costs.

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MATERIAL AND METHODS

The trials were established in the experimental station Lukavec, Czech Republic (49.56N, 14.99E, 610 m a.s.l., potato cropping area, annual mean temperature 7.3°C, and annual mean precipitation 682 mm), and were running from 2012 to 2016. The soil type at the experimental site was modal, sandy loamy Cambisol with 7° slope. The mean value of the soil pH was 6.1, mean content of the organic carbon was 1.06% and mean content of total nitrogen was 0.18%.

Three different soil cultivation methods were evaluated. The conventional tillage (CT), included ploughing (25 cm), seed-bed preparation, and sowing, which served as a control treatment. No-tillage (NT) method consisted of direct sowing into the untilled soil. The soil surface was covered by mulch from post-harvest residues of the preceding crop when maize emerged. Minimum tillage (MT) included shallow soil loosening to the depth of 15 cm, incorporating stubble residues in the same way. Three times replicated erosion plots with different tillage treatments (3 × 8 m = 24 m^2 – longer side was in the direction of the slope) were bounded with sheet metal strips to measure exactly the amount of precipitation running off from the experimental area. For that reason, containers were installed on the bottom of each erosion plot to collect runoff water and soil particles. Collected water and soil samples were separated, analyzed, and recalculated to one hectare and statistically analyzed (Statistica 12.0, StatSoft) by one-way and multifactorial analysis of variance (ANOVA, MANOVA) and by Tukey's post hoc test. Besides the analysis of naturally induced erosion events (E1-E5), a test of soil infiltration abilities with a rain simulator was conducted. This test was carried out once a year, following the harvest of silage maize.

The rain simulator measurement was carried out on each replication of all tillage treatments at a designated area of 0.5 m^2 from a rain simulator designed by the Research Institute of Agricultural Engineering (Prague-Ruzyně). Surface runoff was measured at a constant operating pressure of 100 kPa from a height of 1 m (Šindelář *et al*. 2007) for 60 minutes.

All post-harvest residues were left on the soil surface on the measured areas. Before sprinkling, the selected areas of measurement were characterized by soil moisture at a depth 10 cm (soil hygroscope Theta HH2), sloping and surface roughness with digital gradiometer (BMI IncliTronic Plus, Germany) according to method Klik *et al.* (2002)

The simulated rain is defined by its intensity and operating time of a simulator, i.e. duration of the rain. The precise amount of water, infiltrating the soil, was calculated by the difference between the simulated precipitation and the amount of cumulative surface water runoff from the experimental plot. The amount of water from the surface runoff was weighted at five-second intervals and the amount of the surface water runoff [m^3/ha] and soil loss [kg/ha] during erosion was recalculated to 1 ha.

RESULTS AND DISCUSSION

Evaluation of erosion events caused by natural rainfall

During the vegetation period of 2012, five erosion events (E1-E5) with precipitation runoff and soil losses were recorded (Figure 1a, 1b). The most erosive effect was found in CT after heavy rains on July 28 (E5), when more than 30 mm of precipitation over two hours, 8.9 m^3 of runoff water and 436.6 kg/ha of soil loss, was recorded. The MT significantly decreased surface water runoff (6.6 m^3/ha) and soil losses (143 kg/ha). The lowest erosion was recorded in NT (water runoff and soil losses were about 60 and 21%, respectively, lower, compared to CT).

The most serious event in 2013 was recorded from 24 to 26 of June (137 mm of precipitation) (E2, Figure 1c, 1d). The highest losses of water (7.52 m^3/ha) and soil (327 kg/ha) were recorded at the CT treatment. In the case of NT and MT, the water runoff was 4.61 m^3/ha and 4.36 m^3/ha, respectively, during E2. Even more significant differences were observed in soil runoff, where the soil losses ranged from 29.3% (NT) to 40.9% (MT) compared to the CT plots.

In 2014, we recorded five erosion events. After less intense rainfall on July 7 (E4), there was surface water and soil runoff found only on the CT plots (Figure 1e, 1f). From the graphs, it is clear that the highest amount of runoff water and soil losses were measured after a hail storm on June 11 (E3), with

53.6 mm of precipitation falling during one day.

The greatest erosion effect was visible on CT plots (10.13 m^3/ha of water runoff and 435 kg/ha of soil losses). On the contrary, the plots with applied conservation tillage technologies, lower values of runoff water, and soil losses were recorded during all the erosion events.

In the dry and warm year of 2015, only one erosion event was recorded (August 18, 83.5 mm of precipitation). Surface runoff water was captured only on the CT plots. At the same time, no erosion was recorded on the NT and MT plots during the same time, confirming their ability of higher water infiltration. There was one erosion event in 2016 (July 15th) when 63.3 mm of precipitation was recorded. The highest water erosion was recorded on CT plots (7.30 m^3/ha) while the values of NT and MT were 81.4 and 85.6% lower (Table 1). In the case of soil erosion, the highest losses were found on CT plot, while NT and MT recorded 73.6% and 63.5% lower losses, comparing to the CT treatment. Similar results published by Franzluebbers (2002), who analyzed the effect of different cropping practices on water damage. He stated that organic soil matter is a key factor in limiting the water runoff surface and soil losses. The effect of different

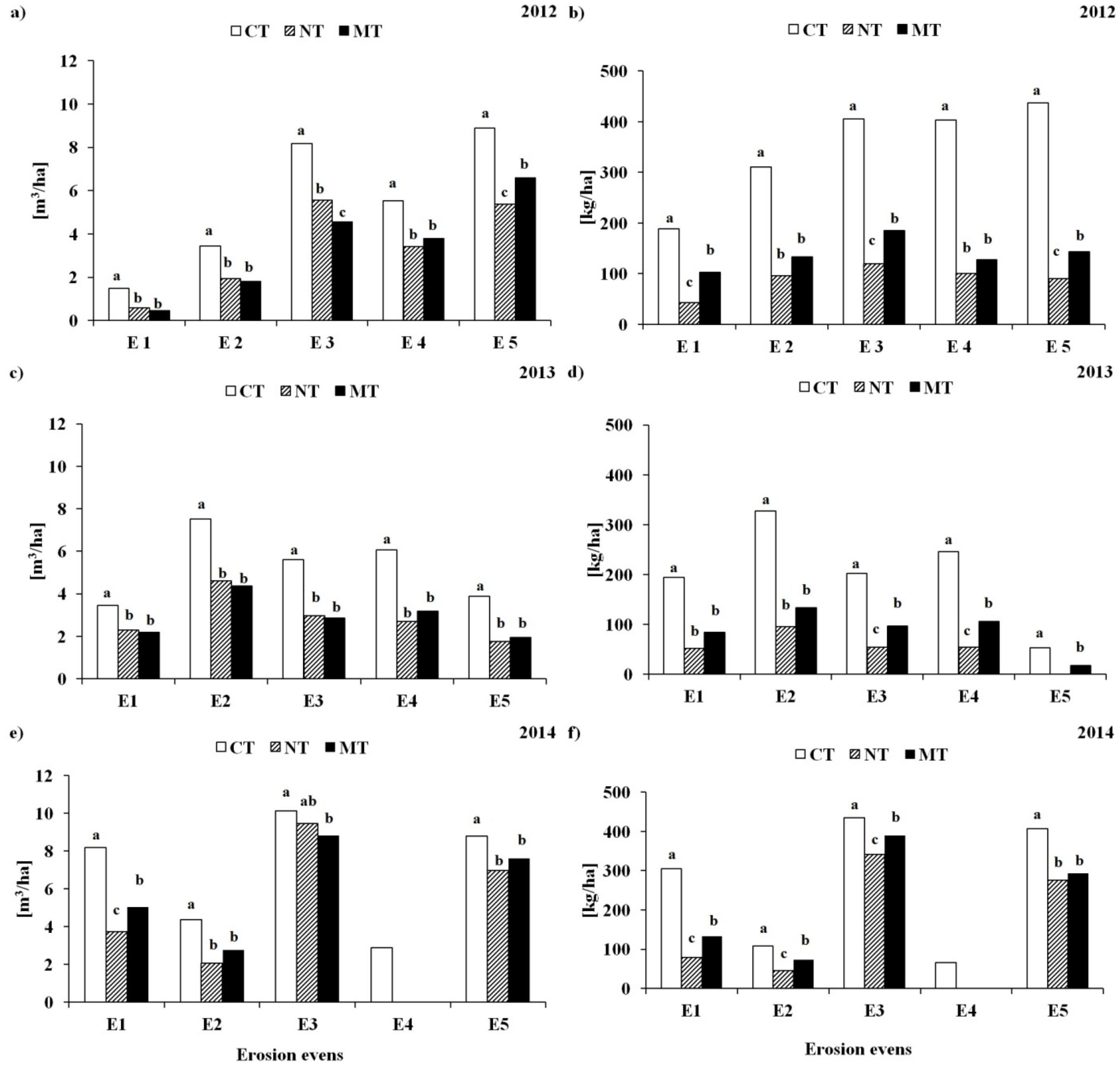

Figure 1. The effect of different intensity and depth of soil tillage (CT – conventional tillage, NT – no tillage, MT – minimum tillage) on surface water runoff in a) 2012, c) 2013 and e) 2014 an on surface soil runoff in b) 2012, d) 2013 and f) 2014

tillage approaches on the ability of topsoil layer to catch rainwater also evaluated by Kovaříček *et al*. (2014). They also recorded the highest erosion control effect in the NT and MT cropping practices and showed that undesirable compaction of the topsoil significantly increases water and soil erosion during the intense erosion events. Also, Govers *et al*. (2017) recommend soil protective tillage approaches (with sequestration of organic carbon into the soil) as a tool of soil protection in areas endangered by erosion.

Evaluation of water erosion caused by simulated rainfall

The time intervals from the beginning of water erosion from the start of the rain simulation and the amount of soil losses from the different tillage treatments in the evaluated period between 2012–2016 are presented in Table 2. From the observed beginning of surface water runoff after simulated rainfall, the NT was evaluated as the most advantageous in terms of reduction of erosion effects. This variant was characterized by the latest onset of the beginning of the surface water runoff practically in all monitored years (in 6.92 minutes in the average of years). Less than two minutes earlier, surface water runoff started on the MT plots. The results obtained clearly showed that the onset of soil erosion was influenced by the mulching of organic matter on the soil surface, which significantly slowed the water runoff and increased the volume of water infiltration into the soil. Only the slightly earlier onset of water runoff (4.71 minutes from the start of simulated rainfall) was found in CT. When simulating the rain in the evaluated treatments on the stubble of the silage maize, the surface layer of the soil was gradually saturated with water, and after the pores were filled, the surface runoff occurred. Water permeability decreased parallel with depth. Under constant rain simulation, the surface runoff increased for an interim period, but after 30 to 45 minutes it stabilized on the difference between the sprinkling intensity and the rate of infiltration into the subsoil (Kovaříček *et al.* 2008). In our experiment, we confirmed the general trend that, the sooner the surface runoff began, the more the soil was washed away. The lowest soil losses (25.37 g/m^2/h), in the average of years, was recorded on the NT plots, while the highest losses (63.67 g/m^2/h) were found on the CT plots (Table 2). Table 3 shows

Table 1

Amount of surface water runoff and soil losses during erosion event in 2016

Soil tillage treatment	Surface water runoff		Soil losses	
	[m^3/ha]	Soil tillage impact [%]	[kg/ha]	Soil tillage impact [%]
CT 1	7.98	–	197.5	–
CT 2	6.93	–	180.3	–
CT 3	6.98	–	157.2	–
Average CT	7.30±0.34^a	100.0	178.3±11.68^a	100.0
NT 1	6.66	–	120.3	–
NT 2	5.68	–	108.5	–
NT 3	5.47	–	110.7	–
Average NT	5.94±0.37^a	81.4	113.2±3.62^b	63.5
MT 1	6.92	–	138.3	–
MT 2	6.08	–	130.6	–
MT 3	5.77	–	124.8	–
Average MT	6.25±0.34^a	85.6	131.2±3.91^b	73.6

Legend: CT – conventional soil tillage; NT – sowing into no-tilled soil; covered with mulch; MT – minimum soil tillage; 1, 2, 3 – replications
Means with standard errors of the mean (SE) followed by the same letter were not significantly different at 0.05 probability level

the results of the year 2014, as the typical and characteristic example.

Results provided evidence that in the case of severe rainfall, the NT and MT significantly reduce surface water runoff, soil losses, and improve rainwater infiltration into the soil.

Evaluation of soil tillage methods on maize yield and financial costs

Soil tillage methods do not only affect erosion, but also the yield of cultivated plants and financial costs. As our experiment lasted from 2013 to 2016, weather conditions of each year significantly affect-

Table 2

Beginning of surface water runoff and soil losses during erosion events in 2012–2016

Tillage treatment	Year	Beginning of surface water runoff [min]		Soil losses due to water runoff [g/m²/h]	
		Value	Average	Value	Average
CT	2012	5.03	4.71	50.64	63.67
	2013	5.38		77.36	
	2014	4.22		52.53	
	2015	4.65		56.34	
	2016	4.27		81.48	
NT	2012	7.28	6.92	18.90	25.37
	2013	7.12		18.37	
	2014	6.58		14.14	
	2015	6.83		24.04	
	2016	6.79		51.41	
MT	2012	6.16	5.08	63.37	48.16
	2013	5.42		30.18	
	2014	4.28		55.71	
	2015	4.97		28.09	
	2016	4.57		63.45	

Table 3

Cumulative surface water runoff from the test area measured in the intervals of 15 minutes under the constant intensity of simulated rain 87 mm per hour (2014)

Tillage treatment	Variant/ replication	Cumulative surface water runoff [l] per given time interval							
		15 min		30 min		45 min		60 min	
		Value	Average	Value	Average	Value	Average	Value	Average
CT	CT 1	2.78	4.86	12.85	14.41	22.92	23.74	29.52	30.58
	CT 2	5.83		16.69		27.63		34.80	
	CT 3	5.98		13.69		20.66		27.41	
NT	NT 1	0.59	1.31	7.54	8.35	15.22	14.83	17.92	19.28
	NT 2	0.65		6.79		9.36		13.71	
	NT 3	2.68		10.71		19.92		26.22	
MT	MT 1	2.77	3.84	10.10	11.27	17.61	19.07	24.20	25.11
	MT 2	2.85		7.88		15.46		19.54	
	MT 3	5.89		15.82		24.13		31.58	

Table 4

The effect of the year (2013, 2014, 2015 and 2016) and NT, MT and CT on the average yield of maize

Tillage treatment	2013	2014	2015	2016	x̄ ± SE
CT	38.56±0.54a	39.30±0.97a	31.65±0.98a	30.27±0.77a	34.59±1.26A
NT	36.55±0.81a	43,77±1.07b	34.79±1.06ab	33.94±0.95a	37.26±1.24B
MT	42.37±1.16b	45.15±0.85b	37.33±1.11b	31.66±0.85a	39.13±1.61C
x̄ ± SE	39.16±0.96C	42.77±1.01D	34.59±0.98B	31.96±0.69A	–

Means with standard errors of the mean with the same letter (small letters vertically, big letters for the means of the year and technology) are not significantly different at 0.05 probability level.

Table 5

Economic evaluation of CT, NT and MT (€/ha)

Soil treatment	Material costs	Costs of mechanization + fixed costs	Total costs	Diesel consumption [l/ha]	Workload [h/ha]
CT	497	466	963	73.8	5.75
NT	609	421	1,030	54.3	5.04
MT	602	425	1,027	49.2	4.18

ed maize yield (Table 4). According to MANOVA, the "year" factor affected yields of maize by 76%, while "tillage system" influenced maize yield only by 19%. The MT (39.13 t/ha) was the most productive system with significantly higher yields than NT (37.26 t/ha) and CT (34.59 t/ha, Table 4). The difference in yields between NT and CT was also significant. The reason for higher grain yields in MT and NT systems is that these approaches positively affect water regime and help to resist weather fluctuation during the season. The same results were published by Mikanová *et al.* (2012), who studied different tillage and its impact on yield of cereals. Håkansson (2005) outlined that soil-protected tillage can also positively affect soil properties, such as bulk density or porosity, accumulation of soil organic matter, and less mobile nutrients in topsoil layer. The economic evaluation of CT, NT, and MT for silage maize was based on our previous experiences from evaluation of winter wheat, spring barley and white mustard (Vach *et al.* 2016), on "Normatives of agricultural production technologies" (Kavka *et al*. 2006) and the normatives of the cropping technologies (www.agronormativy.cz).

In the case of MT and NT, compared to the CT, higher costs for inputs (mainly pesticides) were found. On the other hand, the cost of mechanization was lower, as the number of input measures was reduced (Table 5). This is due to the need of more exacting operations in CT, especially cost of medium deep ploughing (22 cm). It can be stated that with decreasing intensity of soil cultivation, the cost of mechanization can be reduced parallelly.

However, material inputs can significantly increase with reduced processing due to higher pesticides consumption.

CONCLUSIONS

According to the results obtained, we can confirm that NT and MT provided the best erosion control results. On the other hand, CT was evaluated as the method increasing the risk of erosion. In this way, higher erosion control effects of NT and MT were verified by statistically significant higher water infiltration into the soil and significantly lower surface water and soil runoff. The results of this study confirmed the legitimacy for the inclusion of conservation soil tillage in the system of erosion control measures and soil protection against damage by surface runoff of rainwater in eroded areas. At the same time, they reduce the losses of the most fertile soil layers and applied agrochemicals and thus reduce subsequent pollution of the surface and groundwater. NT and MT can better processes soil water regime, positively affecting soil properties, keep soil organic matter and less mobile nutrients in the topsoil layer, positively influencing the yield of crops. These tillage approaches are also cheaper in the case of mechanization, but more expensive for pesticides. When choosing a method of tillage, approaches with less soil cultivation should be preferred not to only reduce costs, but to simplify workload and decrease erosion.

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REFERENCES

BASIĆ, F. – KISIĆ, I. – MESIĆ, M. – NESTROY, O. – BUTORAC, A. 2004. Tillage and crop management effects on soil erosion in central Croatia. In *Soil and Tillage Research*, vol. *78,* no. 2, pp. 197–206.

BHATT, R. – KHERA, K.L. 2006. Effect of tillage and mode of straw mulch application on soil erosion in the submontaneous tract of Punjab, India. In *Soil and Tillage Research*, vol. *88*, pp.107–115.

FRANZLUEBBERS, A.J. 2002. Water infiltration and soil structure related to organic matterand its stratification with depth. In *Soil and Tillage Research*, vol. *66,* no. 2, pp. 197–205.

GOVERS, G. – MERCKX, R. – VAN WESEMAEL, B. – VAN OOST, K. 2017. Soil conservation in the 21st century: why we need smart agricultural intensification. In *Soil*, vol. *3*, pp. 45–59.

HÅKANSSON, I. 2005. Machinery-induced compaction of arable soils. Incidence – consequences – counter-measures. Reports from the division of soil management. No. 109, Sweden, Uppsala, 153 p.

HERNANZ, J.L. – LÓPEZ, R. – NAVARRETE, L. – SÁNCHEZ-GIRÓN, V. 2002. Long-term effects of tillage systems and rotations on soil structural stability and organic carbon stratification in semiarid central Spain. In *Soil and Tillage Research*, vol. *66*, pp. 129–141.

HOLÝ, M. 1994. *Eroze a životní prostředí* [*Erosion and environment*]. Prague: CVUT publishing, 383 pp. ISBN 80-01-01078-3

KAVKA, M. et al. 2006. *Normativy zemědělských výrobních technogií* [Normatives of agricultural production technologies]. ÚZPI Prague (in Czech), 376 pp.

KELLER, T. – LAMANDE, M. – PETH, S. – BERLI, M. – DELENNE, J.Y. – BAUMGARTEN, W. – RABBEL, W. – RADJAI, F. – RAJCHENBACH, J. – SELVADURAI, A.P.S. – OR, D. 2013. An interdisciplinary approach towards improved understanding of soil deformation during compaction. In *Soil and Tillage Research*, vol. *128*, pp. 61–80.

KLIK, A. – KAITANA, R. – BADRAOUI, M. 2002. Desertification hazard in a mountainous ecosystem in the High Atlas region, Marocco. In YUREN, J. (Ed.) *Proceedings from 12th ISCO Conference*. Beijing, China, pp. 636–644.

KOVAŘÍČEK, P. – ŠINDELÁŘ, R. – HŮLA, J. – HONZÍK, I. 2008. Measurement of water infiltration in soil using the rain simulation method. In *Research in Agricultural Engineering*, vol. *54*, no. 3, pp. 123–129.

KOVAŘÍČEK, P. – HŮLA, J. – ABRHAM, Z. – VLÁŠKOVÁ, M. 2014. *Systém hospodaření s cílem omezit nežádoucí zhutnění půdy a zvýšit propustnost půdy pro vodu* [*Farming system to reduce unwanted soil compaction and increase water permeability*]. Applied certified methodology. Prague: RIE Publishing, pp. 40. ISBN 978-80-86884-78-3

MARTENS, D.A. 2000. Management and crop residue influence soil aggregate stability. In *Journal of Environmental Quality,* vol. *29*, pp.723–727.

MIKANOVÁ, O. – JAVŮREK, M. – ŠIMON, T. – FRIEDLOVÁ, M. – VACH, M. 2009. The effect of tillage systems on some microbial characteristics. In *Soil and Tillage Research*, vol. *105*, pp. 72–76. DOI:10.1016/j.still.2009.05.010

MIKANOVÁ, O. – JAVŮREK, M. – ŠIMON, T. – VACH, M. 2012. Relationship between winter wheat yields and soil carbon under various tillage systems. In *Plant, Soil & Environment*, vol. *58,* no. 12, pp. 540–544.

MORGAN, R.P.C. 2005. *Soil Erosion and Conservation*. London: Blackwell Science Publishing, 304 pp. ISBN 978-1-4051-1781-4

SAINJU, U.M. 2002. Long-term effects of tillage, cover crops, and nitrogen fertilization on organic carbon and nitrogen concentrations in sandy loam soils in Georgia, USA. In *Soil and Tillage Research*, vol. *63*, pp. 167–179.

SCHULLER, P. – WALLING, D.E. – SEPÚLVEDA, A. – CASTILLO, A. – PINO, I. 2007. Changes in soil erosion associated with the shift from conventional tillage to a no tillage system, documented using ^{137}Cs measurements. In *Soil and Tillage Research*, vol. *94*, pp.183–192.

ŠIMON, T. – JAVŮREK, M. – MIKANOVÁ, O. – VACH, M. 2009. The influence of tillage systems on soil organic matter and soil hydrophobicity. In *Soil and Tillage Research*, vol. *105*, pp. 44–48. DOI: 10.1016/j.still.2009.05.004

ŠINDELÁŘ, R. – KOVAŘÍČEK, P. – KROULÍK, M. – HŮLA, J. 2007. *Hodnocení povrchového odtoku vody metodou simulace deště* [*Evaluation of surface runoff by the method of rain simulation*], vol. *5*, pp. 1–7. Available on: http://www.agritech.cz/clanky/2007-2-5, pdf

TRAUMAN, C.C. – SHAW, J.N. – REEVES, D.W. 2005. Tillage effects on rainfall partitioning and sediment yield from anultisol in central Alabama. In *Soil Water Conservation*, vol. *60*, pp.89–98.

TEBRÜGGE, F. – DÜRING, R.-A. 1999. Reducing tillage intensity: a review of results a long-term study in Germany. In *Soil and Tillage Research,* vol. *53*, pp.15–28.

VACH, M. – STRAŠIL, Z. – JAVŮREK, M. 2016. Economic efficiency of selected crops, cultivated under different technology of soil tillage. In *Scientia Agriculturae Bohemica*, vol. *47*, pp. 40–46.

ZHANG, G.S. – CHAN, K.Y. – OATES, A. – HEENAN, D.P. – HUANG, G.B. 2007. Relationship between soil structure and runoff soil loss after 24 years of conservation tillage. In *Soil and Tillage Research*, vol. *92*, pp.122–128.

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WINTER BARLEY PRODUCTION IN RELATION TO CROP ROTATIONS, FERTILISATION AND WEATHER CONDITIONS

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The aim of the study was to evaluate the influence of crop rotations, fertilisation and weather conditions on the grain yield and yield components of winter barley. Long-term field experiment was situated in the area of continental climate. There were the crop rotations with 40%, 60% and 80% share of the cereals. Two treatments of fertilisation were applied: H1 and H2. In the first treatment (H1), nitrogen, phosphorus and potassium in mineral fertilisers + organic manure Veget® were applied. In the second treatment (H2), nitrogen, phosphorus and potassium were applied in fertilisers only. Winter barley cultivar Barcelona was grown in years 2010–2015. The number of spikes per 1 m^2, proportion of grains over sieve 2.5 + 2.8 mm and grain yield of winter barley were statistically significantly influenced by fertilisation. In the variant H1 (mineral fertilisation with addition of organic manure Veget®), the statistically higher ($P \leq 0.05$) grain yield was obtained (5.71 t/ha) than in the treatment with mineral fertilisation only (5.30 t/ha). In the year 2011, which was very unfavourable for winter barley development, the grain yield in the treatment with mineral fertilisation with the addition of organic manure Veget® was by 1.23 t/ha (32.1%) higher than that in treatment with mineral fertilisation only.

Key words: winter barley, crop rotation, fertilisation, grain yield, yield components

Nowadays, crop rotations, in general, are considered to be key sustainable practice that influences production ability and maintenance or enhancement of soil fertility in long-term view. Economic conditions are forcing farmers to look at the profit and this leads them to cereal-dominated crop rotations with increasing risk because of unfavourable preceding crops (Sieling & Christen 2015). Jensen *et al*. (2004) carried out the study to compare preceding crops lupine, pea and oat and their effects on the yield of subsequent winter barley crops. The effect of lupine on the subsequent winter barley grain yield was significantly higher than that of pea, probably due to greater N-root nitrogen residues. Besides the balanced crop rotation, successful growth of winter barley is supported by balanced fertilisation. Sieling *et al*. (2014) compared the utilisation of nitrogen from slurries and from mineral fertilisers. In plots without N fertilisation, oilseed rape showed no yield trends during the experimental period, whereas wheat (barley) yield started to decrease after 10 (13) years without N fertilisation. Possibilities of the application of organic-mineral fertilisers in various forms and their importance for yield stabilisation are investigated. Studies dealing with winter barley nutrition, in general, report about controlled nutrition based on soil analyses of the phosphorus and potassium and on soil and plant analyses of the nitrogen (Molnárová *et al.* 2012). Organic manure is the source of organic matter and nutrients. However, these nutrients are gradually released and it takes time to integrate them back to the soil–plant system. Fertilisers provide nutrients that can be relatively quickly absorbed by plants, leading to the develop-

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ment of a rich root system and thus higher yields under good weather conditions (Hejcman *et al.* 2012). In dry seasons, the mineralisation of fertiliser is decreased, and in wet seasons when the soil moisture is near the field capacity, the mineralisation is increased (Cassman & Munns 1980). Whilst regular application of high rates of mineral nutrients can provide high yields and leave a relatively good pool of nutrients in the soil, organic manure release more nutrients (N, P, K, Ca, Mg and others) in smaller amount and for a longer period. Thus combined application of mineral fertiliser with nitrogen, phosphorus, potassium (NPK) and farmyard manure (FYM) provides a wide range of nutrients that are released in both short and long period and reduces the negative effect of mineral fertiliser NPK on topsoil pH (Hlisnikovský & Kunzová 2014).

Winter barley has a rapid initial growth and good nutrients utilisation. The differences in dissimilar grain yield development between wheat and barley are explained by the different efficiency of nitrogen and water utilisation (Cossani *et al.* 2012). Barczak (2008) took samples from winter barley in five growth stages from the tillering to ripening: tillering, stem elongation, heading, grain filling and wax ripening. The results revealed that the content of N, P, K, Ca, Mg and Na in the above ground biomass of winter barley decreased in subsequent phenological stages from tillering up to wax ripening. In general, nitrogen had a positive effect on the content of monitored microelements in the above ground biomass of winter barley in all growth stages. Charles *et al.* (2012) compared two-row and six-row varieties of winter barley. They found out that higher grain yield of six-row variety was caused by producing a higher number of grains in spike. The better tillering and higher weight of grain in the two-row variety were not able to compensate the higher number of grains in spike in the six-row variety. Two-row variety showed higher protein, fat and mineral substances in grain. These parameters were influenced by nitrogen fertilisation.

The objective of this study was to evaluate the effect of two different crop rotations, fertilisation and climate conditions in years 2010–2015 on the number of spikes per square metre, bulk density of grains, 1,000 grains' weight, the proportion of grain higher fractions (2.8 + 2.5 mm) and grain yield of winter barley.

MATERIAL AND METHODS

The presented results are part of a long-term field experiment established in 1974 in the maize-barley-producing area at the Experimental Station Borovce, belonging to the National Agricultural and Food Centre – Research Institute of Plant Production. The experimental fields at Borovce are located at an elevation of 167 m a.s.l. The region is characterised by continental climate with average annual rainfall of 593 mm (from 358 mm during the vegetation period) and long-term average annual temperature of 9.2°C (15.5°C during the vegetation season). The climatic conditions in Borovce are presented in Tables 1 and 2. The soil characteristics of field plots were as follows: Luvi-haplic Chernozem. The depth of the topsoil layer was 24–28 cm. The content of nutrients in the soil is given in Tables 3 and 4 (the analysis was according to Mehlich II). The rates of nutrients applied in fertilisers are in Tables 5 and 6. Conventional ploughing to a depth of 22–25 cm, sowing, fertilisation and plant treatments were performed according to the recommendations for the given area (the term of sowing: at least 10th of October; sowing rate: 450 seeds/1 m^2). The pesticides were used according to the actual occurrence of diseases, pests and weeds. The winter barley was grown in crop rotations with 60% and 80% proportion of cereals (Table 7). In both crop rotations, the preceding crop of winter barley was winter wheat. In crop rotations, two different fertilisation of winter barley were used: H1 – mineral fertilisation + organic manure Veget®; H2 – only fertiliser amendment. The mineral fertilisation consisted of N, P and K, application rates of P and K was calculated according to the balance methodology (Bizík *et al.* 1998). Veget® was applied at a dose of 5 tons/ha/year. The composition of organic manure Veget® was as follows: dry matter content minimum, 85% (includes combustible matter content 75%); total N content, 2.5–3.0%; total P_2O_5 content, 0.5–2.0%; K_2O content, 1.5%; C:N ratio, 13:1; and pH (in water), 8.5. Total N rates applied to winter barley were split to basic fertilisation – in autumn before sowing and regenerative fertilising – very soon in spring. In the experimental years 2010–2015, the winter barley cv. Barcelona (double row cultivar) was used.

Table 1

Weather conditions in the experimental years 2010–2012 on the stand Borovce

Month	n (1951–1980)		2010		2011		2012	
	x_{td} [°C]	∑ [mm]	x_{td} [°C]	∑ [mm]	x_{td} [°C]	∑ [mm]	x_{td} [°C]	∑ [mm]
January	–1.8	32	2.22	60.6	–1.87	32.4	–0.48	78.8
February	0.2	33	1.39	38.0	–2.06	8.0	–4.59	39.2
March	4.2	32	4.82	19.5	4.31	29.0	5.07	4.5
April	9.4	43	9.91	65.0	11.6	30.4	9.07	20.3
May	14.1	54	15.41	168.3	14.68	93.2	15.43	16.2
June	17.7	80	19.47	95.0	19.15	165.2	18.88	108.1
July	18.9	76	23.02	98.0	18.14	83.2	20.73	94.1
August	18.4	68	19.65	99.5	20.48	25.4	20.12	10.6
September	14.5	38	13.42	101.5	16.41	17.8	14.76	41.5
October	9.6	42	8.04	25.0	7.77	32.9	8.02	88.5
November	4.6	51	7.36	76.0	1.37	2.0	4.88	22.6
December	0.3	46	–2.23	48.8	0.03	42.4	–2.81	46.5
x_{td} [ºC]	9.2	–	9.84	–	9.17	–	9.09	–
∑ [mm]	–	595	–	895.2	–	561.9	–	570.9

N – long-term (30-year) normal; x_{td} – average air temperature; ∑ – sum of precipitation

Table 2

Weather conditions in the experimental years 2013–2015 on the stand Borovce

Month	n (1951–1980)		2013		2014		2015	
	x_{td} [°C]	∑ [mm]	x_{td} [°C]	∑ [mm]	x_{td} [°C]	∑ [mm]	x_{td} [°C]	∑ [mm]
January	−1.8	32	−2.55	69.8	−0.21	34.4	−1.29	64.5
February	0.2	33	−0.77	90.3	1.22	33.2	−1.51	28.9
March	4.2	32	0.84	75.3	6.14	20.7	2.71	53.1
April	9.4	43	9.15	17.4	9.65	65.7	8.17	21.9
May	14.1	54	13.52	67.4	13.16	110.3	13.27	58.9
June	17.7	80	17.51	70.1	18.0	34.5	18.34	21.0
July	18.9	76	20.71	3.0	19.85	120.1	22.28	24.8
August	18.4	68	20.12	112.9	17.12	50.9	21.94	111.0
September	14.5	38	11.59	75.6	14.06	122.9	14.13	47.6
October	9.6	42	8.71	29.1	9.19	53.3	7.12	63.5
November	4.6	51	3.33	59.7	4.78	24.9	3.45	40.5
December	0.3	46	−0.57	9.9	−0.23	49.4	−0.06	22.0
x_{td} [ºC]	9.2	–	8.47	–	9.40	–	9.05	–
∑ [mm]	–	595	–	680.4	–	720.3	–	557.7

N – long-term (30-year) normal; x_{td} – average air temperature; ∑ – sum of precipitation

Table 3

The content of nutrients in soils of experimental field plots during the seasons 2009–2012
(analyses in autumn)

SC	2009/2010			2010/2011			2011/2012		
	P	K	Nan	P	K	Nan	P	K	Nan
	[mg/kg]								
60% H1	112	365	9.13	65	227	6.54	142	308	6.19
60% H2	128	361	8.22	69	230	6.82	137	315	7.05
80% H1	86	335	6.23	102	204	5.96	67	218	2.53
80% H2	91	340	7.18	108	201	5.71	59	221	3.47

SC – share of cereals in crop rotation; Nan – $NH_4^+ + NO_3^-$

Table 4

The content of nutrients in soils of experimental field plots during the seasons 2012–2015
(analyses in autumn)

SC	2012/2013			2013/2014			2014/2015		
	P	K	Nan	P	K	Nan	P	K	Nan
	[mg/kg]								
60% H1	137	301	15.17	169	312	13.1	136	316	8.73
60% H2	134	306	14.85	175	319	15.2	123	307	8.27
80% H1	91	247	11.91	94	248	12.3	129	342	6.29
80% H2	85	241	12.07	82	254	11.2	95	298	6.78

SC – share of cereals in crop rotation; Nan – $NH_4^+ + NO_3^-$

Table 5

Rates of nutrients: nitrogen (N), phosphorus (P) and potassium (K) in 2009–2012

SC	2009/2010				2010/2011				2011/2012			
	Autumn			Spring	Autumn			Spring	Autumn			Spring
	P	K	N	N	P	K	N	N	P	K	N	N
	[kg/ha]											
60% H1	0	0	30	50	0	110	35	50	0	0	30	50
60% H2	0	0	30	50	0	120	35	50	0	0	30	50
80% H1	30	0	35	50	30	110	35	50	35	110	35	50
80% H2	30	0	35	50	30	120	35	50	35	120	35	50

SC – share of cereals in crop rotation

Table 6

Doses of nutrients: nitrogen (N), phosphorus (P) and potassium (K) in 2012–2015

SC	2012/2013				2013/2014				2014/2015			
	Autumn			Spring	Autumn			Spring	Autumn			Spring
	P	K	N	N	P	K	N	N	P	K	N	N
	[kg/ha]											
60% H1	0	0	0	50	0	0	0	50	0	0	30	50
60% H2	0	0	0	50	0	0	0	50	0	0	30	50
80% H1	30	110	15	50	30	110	0	50	0	0	35	50
80% H2	30	120	15	50	30	120	15	50	30	120	35	50

SC – share of cereals in crop rotation

In this work, the bulk density of grains, 1,000 grain's weight, number of spikes per square meter, the proportion of grains over sieve 2.5 + 2.8 mm and grain yield were evaluated.

The statistical significance of the data was computed by multiple-way analysis of variance (ANOVA). In addition, the least significant difference (*LSD*) multiple range test was used to determine which means differed significantly from the others, using a significance level of 0.05%. These analyses were performed using Statistica 6.1. (StatSoft Inc., Tulsa, USA).

RESULTS AND DISCUSSION

The years 2010–2015 were extreme in term of average daily temperatures and monthly sums of rainfall (Tables 1 and 2). In the years 2010–2015, the average daily temperatures were increase in June by 0.9°C and in July by 1.9°C in comparison to the long-term average. In 2010–2015, monthly sum of rainfall in May exceeded long-term average by 32 mm. The increase in monthly sum of rainfall in comparison with long-term average was 114.3 mm in May 2010 39.2 mm in May 2011 and 85.2 mm in June 2011; 43.3 mm in March 2013 and 56.3 mm in May 2014. The weather in the individual years influenced the production ability of winter barley in the biggest measure. The proportion of cereals in crop rotations has no statistically significant influence on yield components and grain yield of winter barley. The combination of mineral fertilisers with organic manure influenced the grain yield and yield components of winter barley in considerable extent, particularly under unfavourable weather conditions in 2011 (exceed of precipitation in May and June; high temperature in April), 2013 (lack of precipitation in April) and 2015 (lack of precipitation in April and June).

Table 7

The crop rotations with 60% and 80% share of the cereals

Crop rotations	
60% share of cereal	80% share of cereal
1. pea	1. winter wheat
2. winter wheat	2. spring barley
3. winter barley	3. pea
4. silage maize	4. winter wheat
5. spring barley	5. winter barley

The bulk density and 1,000 grains' weight were statistically significantly influenced by weather in particular years and interaction between fertilisation and years. In year 2011, the bulk density was statistically lower (651 g/l) than that in 2010, 2012, 2013 and 2015 (Figure 1, Table 8). In year 2011, 1,000 grains' weight was statistically lower (47.5 g) than that in 2010, 2012, 2013, 2014 and 2015 (Figure 2, Table 8). Sieling and Christen (2015) reported that 1,000 grains' weight was decreased by unsuitable preceding crop (wheat).

Weather in particular years and fertilisation had a statistically significant influence on the number of spikes per 1 m^2. In year 2013, the number of spikes per 1 m^2 was statistically significantly higher (745 pcs/m^2) than that in 2010, 2011, 2012 and 2015 (Figure 3, Table 8). In the treatment with mineral fertilisers and organic manure Veget® incorporation, the number of spikes (Table 9) was statistically higher (630 pcs/m^2) than in the treatment with mineral fertilisation only (557 pcs/m^2).

The proportion of grains 2.8 + 2.5 mm of winter barley was statistically significantly influenced by weather in particular years, fertilisation and interaction between fertilisation and years. The share of grains 2.8 + 2.5 mm was statistically higher in 2010 (63.0%) and 2015 (63.6%) than in 2011, 2012, 2013 and 2014 (Figure 4, Table 8). In the treatment with mineral fertilisers and organic manure Veget®, the

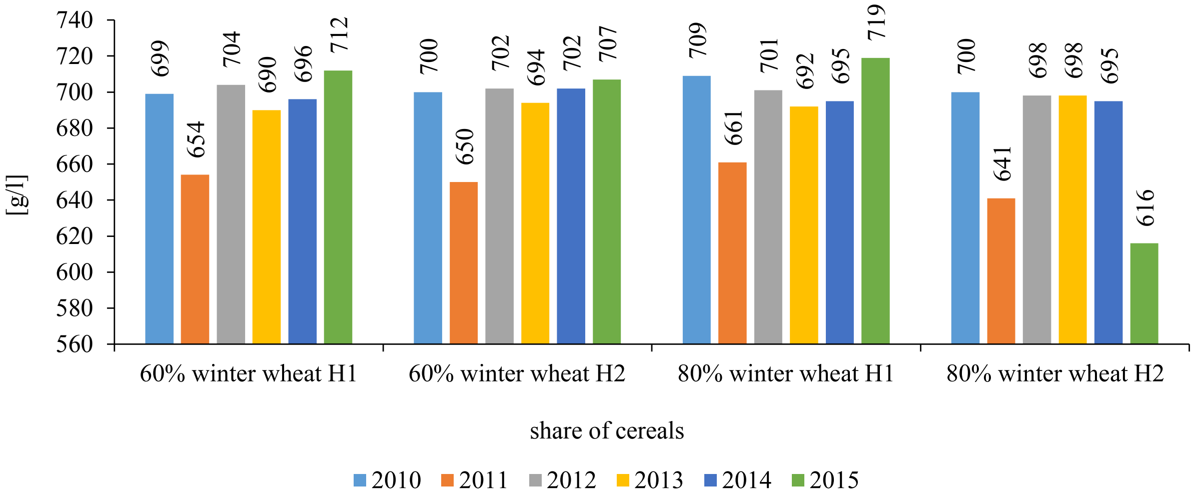

Figure 1. The bulk density [g/l] of winter barley in crop rotations

Table 8

Average values of grain yield and yield components in years 2010–2015

Years	BD [g/l]	OTGW [g]	NS [pcs/m^2]	PGOS [%]	GY [t/ha]
2010	702^b	50.4^b	582^{ab}	63.0^b	5.76^b
2011	651^a	47.5^a	497^a	52.1^a	4.44^a
2012	701^b	52.0^c	426^a	52.1^a	4.56^a
2013	694^b	51.1^{bc}	745^{bc}	51.1^a	5.93^b
2014	697^b	49.3^{bd}	677^b	50.5^a	5.69^b
2015	711^c	50.1^b	632^{bd}	63.6^b	6.63^c
Average	693	50.1	593	55.4	5.50
$LSD_{0.05}$	8.86	1.51	103.71	7.74	0.53

BD – bulk density; OTGW – 1,000 grains' weight; NS – number of spikes; PGOS – proportion of grains over sieve; GY – grain yield

The identical characters or identical group of characters (e.g. bc, bc) mean that there are no statistically significant differences. The different characters or different group of characters (e.g. bc, bd) mean that there are statistically significant differences. If there is identical characters in the group of characters with singly located character (e.g. a, ab or b, bc), there are no statistically significant differences.

share of grains 2.8 + 2.5 mm was statistically higher (58.8%) than that in the treatment with mineral fertilisation only (52.1%) – Table 9.

The grain yield of winter barley was statistically significantly influenced by fertilisation, years and interaction between fertilisation and years. The grain yield was statistically lower in 2011 (4.44 t/ha) and 2012 (4.56 t/ha) than in 2010, 2013, 2014 and 2015 (Figure 5, Table 8). In the treatment with fertilisers and organic manure Veget®, the grain yield was statistically higher (5.71 t/ha) than that in the treatment with mineral fertilisation only (5.30 t/ha) – Table 9. A positive effect of organic manure Veget® on number of ears, share of grain 2.8 + 2.5 explained the increase in winter barley grain yield across experimental years and treatments. In 2010–2015, the difference between average grain yield because of the different fertilisation was 7.7%.

Kismanyoky and Toth (2013) reported that depending on the type of crop, the specific year and the N content in the soil, with combined application of mineral fertilisers and organic manure, resulted in a 12–17% increase of crop yield. In our experiment in 2011, the increase in winter barley grain yield in the treatment with mineral fertilisers and organic manure Veget® incorporation was by 32.1% higher than that in the treatment with mineral fertilisation only.

In accordance with our results, Hernández *et al.* (2013) found out that the nitrogen content in the soil

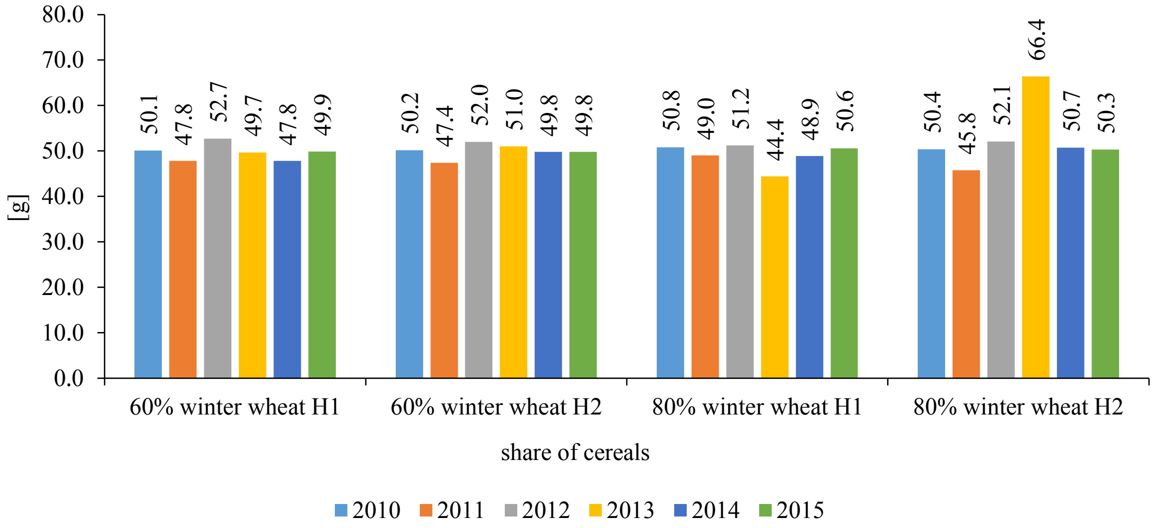

Figure 2. The 1,000 grains' weight [g] of winter barley in crop rotations

Table 9

Average values of grain yield and yield components in two fertilisation treatments

Fertilisation	NS [pcs/m^2]	PGOS [%]	GY [t/ha]
H1	630^a	52.1^a	5.71^a
H2	557^b	58.8^b	5.30^b
Average	557	55.5	5.51
$LSD_{0.05}$	40.7	3.04	0.21

NS – number of spikes; PGOS – proportion of grains over sieve; GY – grain yield
The identical characters mean that there are no statistically significant differences. The different characters mean that there are statistically significant differences.

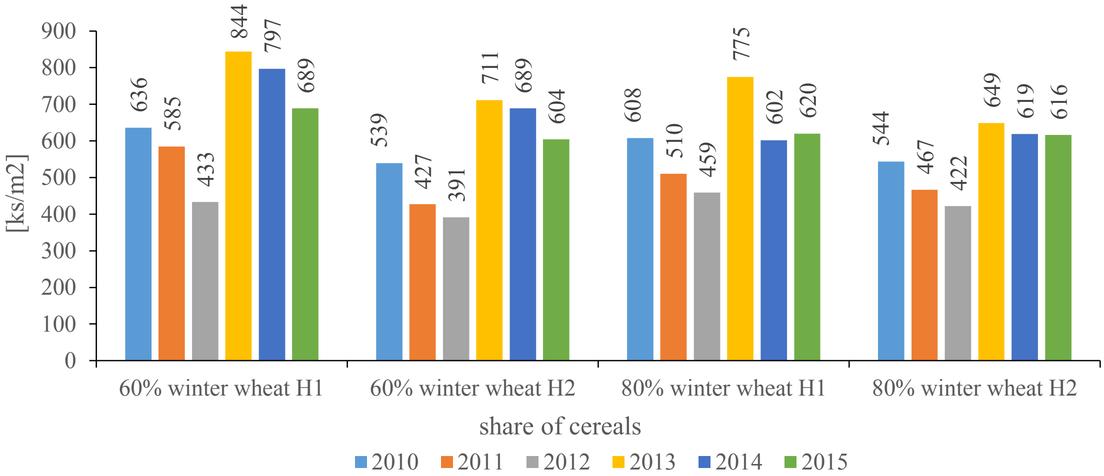

Figure 3. The number of spikes on 1 m² [pcs/m²] of winter barley in crop rotations

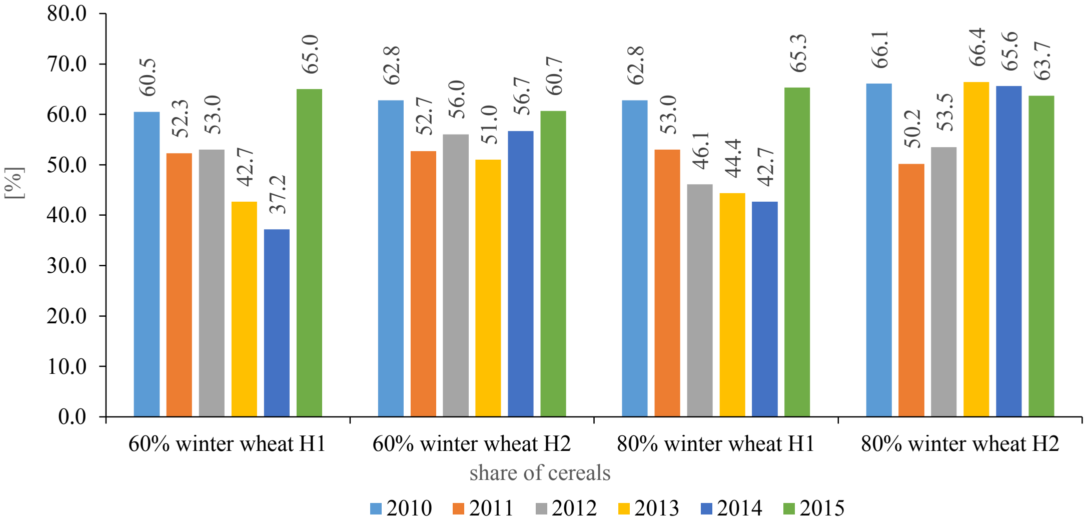

Figure 4. The grain over sieve 2.5 mm [%] of winter barley grain in crop rotations

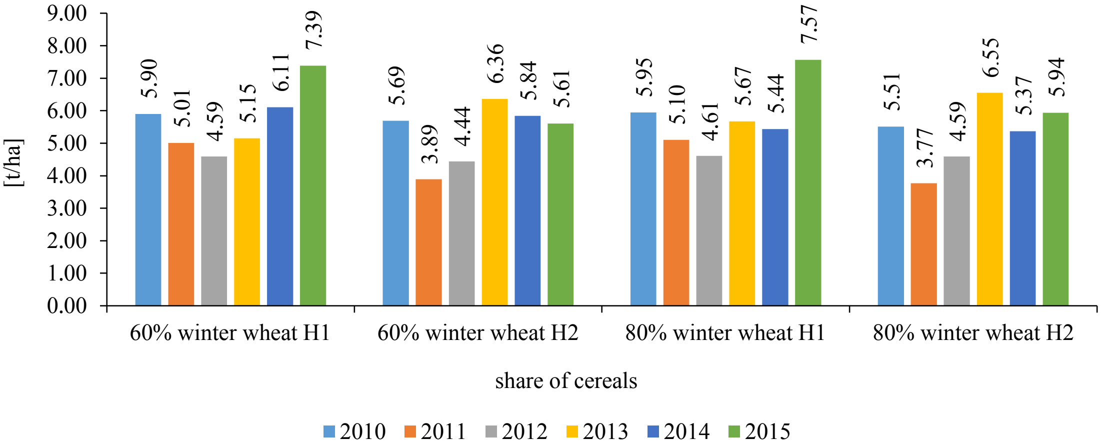

Figure 5. The grain yield [t/ha] of winter barley in crop rotations

for the plants was higher in the treatment with annual pig dung application in comparison with the treatment with mineral fertilisation only or in comparison with untreated plot. These differences were clearly evident in dry years. Plaza-Bonilla *et al.* (2017) found out that for a given N rate (0; 75 kg N/ha; 150 kg N/ha), the use of organic fertilisation significantly increased the water use efficiency in both tillage methods (conventional tillage and no-tillage). Hlisnikovský and Kunzová (2014) reported the results from the long-term field experiment. The field experiment was established in 1954. The effect of six fertiliser treatment on a topsoil pool of nutrient content, organic carbon content (Cox) and pH was evaluated between the years 2001 and 2012. The highest yields were reached in the treatment with the NPK + FYM fertilisation. The application of organic manure, without any mineral fertilisers, cannot provide a sufficient amount of nutrients to create high yields and increase the soil nutrient pool for the next arable crops. Kovacevic *et al.* (2009) investigated the influence of different fertilisation treatments on the grain yield of corn, winter wheat and winter barley. The grain yield of winter barley was increased by 19% in the treatment with higher doses of phosphorus and potassium.

Our results revealed that the increase in grain yield in 2015 was caused mainly by statistically significantly higher number of spikes and the proportion of grains 2.8 + 2.5 mm of winter barley in the treatment with fertilisers and organic manure Veget® incorporation.

Charles *et al*. (2012) emphasised the dependence of winter barley grain yield on increasing fertilisation rates under favourable growing conditions, especially during the period with sufficient amount of accessible water. According to Albrizio *et al.* (2010), water availability enhances N absorption: the response of barley to N fertilisation was higher in the year with better water availability during the most sensitive stages to drought stress. Cantero-Martinez *et al*. (1995) claimed that in very dry year, the higher rate of N fertilisation depressed the yield. Nitrogen fertilisation in very dry season increased green area index and nitrogen uptake by the plant but did not affect any yield component.

In years 2010–2015, we observed an increasing frequency of extreme weather events such as excess of precipitation, lack of precipitation or high temperature. Therefore, searching for arrangements to mitigation of negative impact on the grain yield and yield components is needed. Fernández-Getino *et al.* (2015) estimated the influence of rainfall and temperature on barley yield parameters in different crop and tillage systems. Regarding the influence of previous crop on barley yield, vetch and fallow favourably influenced the winter barley grain yield. The analysis of barley components with minimum tillage and no tillage did not show significant differences compared to conventional tillage. De Vita *et al*. (2017) found that reduced inter-row distance dramatically reduced weed biomass and increased wheat yield and nitrogen uptake. An agronomic improvement in grain yield and quality of winter wheat could be obtained through the application of strategies, such as application foliar fungicides or fertilisers. These treatments protect health of the last leaves and delay the senescence process during ripening (Marinaccio *et al.* 2015).

CONCLUSIONS

The climate in the period 2010–2015 was the strongest factor influencing the grain yield and yield components of winter barley. The differences in grain yields of winter barley depending on the weather in particular years reached 2.19 t/ha (49.3%). Fertilisation is another very important factor influencing the production ability of winter barley. In years 2010–2015, the average grain yield of winter barley in the treatment with mineral fertilisers and organic manure Veget® incorporation was 0.41 t/ha (7.7%), which was higher than that in treatment with mineral fertilisers only. Increasing frequency of extreme weather events such as excessive rainfall and prolonged droughts has been observed. Our study confirmed that the application of organic manure contributes to winter barley yields stabilisation. That was manifested most clearly in year 2011. In this year, the difference in grain yield of winter barley in the treatment with mineral fertilisation and organic manure incorporation was 1.23 t/ha (32.1%), which was higher than that in the treatment with fertilisers only.

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REFERENCES

ALBRIZIO, R. – TODOROVIC, M. – MATIC, T. – STELLACCI, A.M. 2010. Comparing the interactive effect of water and nitrogen on durum wheat and barley grown in a Mediterranean environment. In *Field Crop Research*, vol. *115*, pp. 179–190.

BARCZAK, B. 2008. Contents and ratios of mineral components in winter barley biomass cultivated under conditions of different nitrogen fertilisation. In *Journal of Elementology*, vol. *13*, no. 1, pp. 291–300.

BIZÍK, J. – FECENKO, J. – KOTVAS, F. – LOŽEK. O. 1998. *Metodika hnojenia a výživy rastlín* [Methodology of plant fertilisation and nutrition]. Nitra : SPU, pp. 112. ISBN 80-967812-1-9

CANTERO-MARTINEZ, C. – VILLAR, J.M. – ROMAGOSA, I. – FERERES, E. 1995. Nitrogen fertilization of barley under semi-arid rained conditions. In *European Journal of Agronomy*, vol. *4*, no. 3, pp. 309–316.

CASSMAN, K.G. – MUNNS, D.N. 1980. Nitrogen mineralization as affected by soil moisture, temperature and depth. In *Soil Science Society of America Journal*, vol. *44*, no. 6, pp. 1233–1237. DOI:10.2136/SSSAJ1980.03615995004400060020X

CHARLES, R. – COLLAUD, J.F. – HAENER, L.L. et al. 2012. Varieties, seeding rate and nitrogen fertilization on winter barley. Nitrogen and water use efficiencies of wheat and barley under a Mediterranean environment in Catalonia. In *Agrarforschung Schweiz,* vol. *3*, no. 2, p. 88.

COSSANI, C.M. – SLAFER, G.A. – SAVIN, R. 2012. Nitrogen and water use efficiencies of wheat and barley under a Mediterranean environment in Catalonia. In *Field Crop Research,* vol. *128*, pp. 109–118.

DE VITA, P. – COLECCHIA, S.A. – PECORELLA, I. – SAIA, S. 2017. Reduced inter-row distance improves yield and competition against weeds in a semi-dwarf durum wheat variety. In *European Journal of Agronomy*, vol. *85*, pp. 69–77.

HEJCMAN, M. – KUNZOVÁ, E. – ŠREK, P. 2012. Sustainability of winter wheat production over 50 years of crop rotation and N, P and K fertilizer application on illimerized luvisol in the Czech Republic. In *Field Crop Research*, vol. *44*, pp. 78–86.

FERNÁNDEZ-GETINO, A.P. – SANTÍN-MONTANYÁ, M.I. – ZAMBRANA, E. – ANDRÉS, E.F. – TENORIO, J.L. 2015. The response of barley to rainfall and temperature in different tillage and crop rotation systems in semi-arid conditions. In *Annals of Applied Biology*, vol. *166*, pp. 143–153.

HERNÁNDEZ, D. – POLO, A. – PLAZA, C. 2013. Long-term of pig slurry on barley yield and N use efficiency under semiarid Mediterranean conditions. In *European Journal of Agronomy,* vol. *44*, pp. 78–86.

HLISNIKOVSKÝ, L. – KUNZOVÁ, E. 2014. The content of topsoil nutrients, pH and organic carbon as affected by long-term application of mineral and organic fertilizers. In *Agriculture (Poľnohospodárstvo),* vol. *60*, no. 4, pp. 142–148. DOI: 10.1515/agri-2015-0003

JENSEN, CH.R. – JOERNSGAAD, B. – ANDERSEN, M.N. – CHRISTIANSEN, J.L. –MOGERSEN, V.O. – FRIIS, P. – PETERSEN, C.T. 2004. The effect of lupins as compared peas and oats on subsequent winter barley crop. In *European Journal of Agronomy,* vol. *20*, pp. 405–418.

KISMANYOKY, T. – TOTH, Z. 2013. Effect of mineral and organic fertilization on soil organic carbon content as well as on grain production of cereals in the IOSDV (ILTE) long-term field experiment, Keszthely, Hungary. In *Archives of Agronomy and Soil Science,* vol. *59*, no. 8, pp. 1121–1132.

KOVACEVIC, V. – STOJIC, B. – RASTIJA, M. – BRKIC, I. – DREZNER, G. 2009. Response of maize, wheat and barley to phosphorus and potassium fertilization. In *Cereal Research Communications,* vol. *37*, SUPPL. 1, pp. 129–132.

MARINACCIO, F. – REYNERI, A. – BLANDINO, M. 2015. Enhancing grain yield and quality of winter barley through agronomic strategies to prolong canopy greenness. In *Field Crop Research,* vol. *170*, pp.109–118.

MOLNÁROVA, J. – BREZINA, R. – POSPISIL, R. 2012. Evaluation of variety and fertilization effect on the winter barley yield. In *Agrochémia,* no. 4, pp. 26–29.

PLAZA-BONILLA, D. – CANTINO-MARTINEZ, C. – BARECHE, J. – ARRUE, J.L. – LAMPURLANES, J. – ALVARO-FUENTES, J. 2017. Do no-till and pig slurry application improve barley yield and water and nitrogen use efficiencies in rain Mediterranean conditions? In *Field Crop Research,* vol. *203*, pp. 74–85.

SIELING, K. – CHRISTEN, O. 2015. Crop rotation effects on yield of oilseed rape, wheat and barley and residual effect on the subsequent wheat. In *Archives of Agronomy and Soil Science,* vol. *61*, no. 11, pp.1531–1549.

SIELING, K. – KANG, Ni. – KAGE, H. 2014. Application of pig slurry – First year and residual effects. In *European Journal of Agronomy,* vol. *20*, pp. 405–418.

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