University of Zagreb

FACULTY OF AGRICULTURE

Ivana Šestak

Use of Field Spectroscopy for Assessment of Nitrogen Use Efficiency in Winter Wheat

DOCTORAL THESIS

Zagreb, 2011

Sveučilište u Zagrebu

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Procjena učinkovitosti gnojidbe dušikom spektroskopijom usjeva pšenice

DOKTORSKI RAD

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„Dar mentalne energije dolazi od Boga, vrhunskog bića i ako mi koncentriramo naše misli o toj istini postajemo skladni s ovom velikom moći.“

„The gift of mental power comes from God, Divine Being, and if we concentrate our minds on that truth, we become in tune with this great power. “

Nikola Tesla

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WITHOUT YOU I WOULDN`T BE HERE!

Zagreb, December 2011
Ivana Šestak

ABSTRACT

Key words: *winter wheat, nitrogen fertilization, nitrogen use efficiency, leaf reflectance, vegetation index, neural networks, linear modeling, classification*

The objective of doctoral study was to evaluate the ability of field VNIR spectroscopy to estimate in-season N status and harvest variables of winter wheat under field conditions, and to determine the effects of N fertilization on spectral reflectance and agronomic characteristics. Agronomic data included leaf total N content, CCI, grain total N content, yield and NUE. Research was conducted on experimental field in Western Pannonian subregion of Croatia. To simulate different crop growth scenarios, field measurements with reflectance (350-1050 nm) were acquired from winter wheat flag leaves grown under different mineral N fertilization treatments ranging from 0 to 300 kg N ha^{-1}, during stem extension (F8) and heading stage (F10.5) of growing period 2008 with cultivar “Fiesta” and 2010 with cultivar “Lucija”. Linear statistical models (SLR, MLR, PLSR), non-linear pattern analysis (ANN) and classification analysis (DA, CLA, ANN) were generated to estimate crop biophysical variables and to discriminate between nitrogen fertilization categories, based on the 1st derivative of reflectance in form of PCs and VIs. N fertilization and high level of greenness during F8 reduced visible and increased NIR spectra. These changes were related to lower levels of chlorophyll and earlier senescence in the N-limited plots and F10.5 stage. The greatest spectral differences between N fertilization treatments and the highest correlations with the winter wheat variables were found in the visible and the red edge region which contributed most to the PC development. High and robust correlations between VIs calculated from reflectance that was acquired at F8-“Lucija”, and all crop variables among each other proved to choose yield [r (NDVI : yield) = 0.91] as input to prediction models development. The absence of treatment x cultivar/year interaction for leaf TN content measured at F8 which indicates possibility for spectral sensing of winter wheat in stem extension stage regardless of cultivar differences. MLR identified a 7PC leaf reflectance model that explained 83 % of the variability in winter wheat yield, and accounted for a large variance in leaf and grain biochemical concentration

(R^2 ~ 0.8, $P < 0.05$) (F8, cultivar „Lucija“). Forecasting NUE from harvest data estimated by ground-based remote sensing was feasible (*r* for observed vs. predicted NUE was 0.81, $p < 0.05$). This relationship is the most encouraging result due to the high predictive ability of the models based on F8 spectral data to accurately estimate winter wheat harvest variables. ANN models were the most efficient in capturing the complex link between yield and leaf reflectance spectra (train and test dataset with r = 0.95 and r = 0.92, RMSEC = 2.57 dt ha^{-1} and RMSEP = 4.41 dt ha^{-1}, respectively) compared to corresponding SLR-VIs, MLR and PLSR models. Performance of the 8 factor PLSR model indicated the highest consistency due to the small difference between RMSEC (4.10 dt ha^{-1}) and RMSEP (4.61 dt ha^{-1}) besides high prediction ability (validation R^2 = 0.84), and showed that it is possible to predict grain yield using hyperspectral field spectroscopy data. Still, NDVI and RVI reached a very strong relationship with yield due to the cross-validation results: R^2 = 0.80 and R^2 = 0.73, respectively. Classification analysis indicated that hyperspectral measurements are best at detecting where N is limited rather than where it is in excess during the fast vegetation development (F8) which is of great interest in precision agriculture by optimizing N top-dressing, identifying crop stress patterns and aid in yield forecasting. Irrespective of pre-analysis type of classification approaches, extreme groups were completely separable [I (Control, N_0) and III (N_{250}, N_{250} + amendments, N_{300})], and the small proportion of variability in intermediate N non-limited treatments [II (N_{100}, N_{150}, N_{200})] was probably result of some additional factors during the growing season. The results obtained in this doctoral thesis confirm the high information potential and feasibility of field spectroscopy for estimation of winter wheat conditions during development and harvest because they are scalable and applicable in high range of N stress and non-N-limited environments. Key spectral features and algorithms should help to support non-destructive and real-time monitoring of N status in wheat production by using hyperspectral remote sensing. Further efforts should be taken to increase the amount, complexity and representation of samples so that the derived model can be applied under varied conditions.

PROŠIRENI SAŽETAK

Procjena učinkovitosti gnojidbe dušikom spektroskopijom usjeva pšenice

Ključne riječi: *ozima pšenica, dušična gnojidba, učinkovitost gnojidbe dušikom, spektralna refleksija lista, vegetacijski indeks, neuralne mreže, linearno modeliranje, klasifikacija*

UVOD

Učinkovita gnojidba dušikom (N) od presudne je važnosti za ekonomski isplativu proizvodnju žitarica i zaštitu okoliša. Razumijevanje procesa koji upravljaju usvajanjem dušika i njegovom distribucijom u biljci ozime pšenice jednako je važno i s obzirom na zaštitu okoliša i na kvalitetu poljoprivrednih proizvoda. Budući da biljka pšenice iskoristi samo oko trećinu primijenjenog dušika tijekom vegetacijskog razdoblja, važno je usmjeravati gospodarenje u ratarskoj proizvodnji prema optimalnoj primjeni mineralnih gnojiva, smanjenju troškova proizvodnje i poboljšanju učinkovitosti usvajanja, akumulacije i iskorištenja dušika u pšenici. Istraživanje obuhvaćeno ovim doktorskim radom ima cilj procijeniti stanje dušika u ozimoj pšenici tijekom vegetacije kao i prinos, te učinkovitost gnojidbe dušikom pod utjecajem različitih količina mineralnog dušičnog gnojiva, koristeći nedestruktivne metode. U svrhu povećanja agronomske učinkovitosti gnojidbe dušikom (NUE – „nitrogen use efficiency“) u uzgoju ozime pšenice sve se više ističe potreba za informacijama o sezonskim promjenama u iskorištenju N u biljci. Poljoprivrednici kao i prateća industrija te korisnici ciljaju na realizaciju većeg profita prateći stanje usjeva prije žetve. Napredna tehnologija kao što je hiperspektralna spektroskopija u vidljivom i blisko-infracrvenom području spektra (VNIR – „visible and near infrared”) u okviru precizne poljoprivrede može unaprijediti gospodarenje dušikom u agroekosustavu. Senzori ovakve vrste koriste refleksiju elektromagnetskog zračenja s vegetacije u određivanju ishranjenosti biljke dušikom te prate razvoj usjeva tijekom cijele vegetacije. U ovom istraživanju, terenska spektroskopija je korištena za modeliranje agronomskih značajki ozime pšenice. Kvantitativna analiza s kalibracijom i validacijom te pratećom statistikom provedena je s ciljem procjene predikcijske i klasifikacijske sposobnosti VNIR spektroskopije u određivanju statusa N u ozimoj pšenici i prognozi prinosa te učinkovitosti gnojidbe dušikom. Primjena daljinskih istraživanja u agronomiji u obliku satelitskih i zračnih snimaka, kao i terenske spektroskopije izuzetno je napredovala zadnjih nekoliko desetljeća. Dosadašnja istraživanja potvrđuju uspješnost korištenja ovakvog senzorskog i nedestruktivnog pristupa u praćenju razvoja usjeva na temelju povezanosti optičkih svojstava vegetacije i određenih biokemijskih i agronomskih varijabli (sadržaj N i klorofila u listu, sadržaj N u zrnu, prinos, NUE), no i dalje postoji potreba za mnogo preciznijom kvantitativnom procjenom razvoja usjeva važnom za učinkovito gospodarenje dušikom u agronomiji. Biljni materijal ima jedinstveni spektralni otisak koji se mijenja pod utjecajem fenoloških promjena tijekom vegetacijskog razdoblja, kao dehidracije ili slabe ishranjenosti dušikom. Terenska spektroskopija, kao vrsta hiperspektralnog daljinskog istraživanja, predstavlja visoko osjetljivu tehniku s mogućnošću kontinuirane kvantitativne procjene stanja N u biljci i prinosa na brz, nedestruktivan i jeftin način, primjenom različitih statističkih modela, zamjenjujući na taj način skupe i dugotrajne laboratorijske analize. Komercijalna primjena spektralne refleksije vegetacije prihvaćena je u okviru „variable rate" tehnologije (VRT), odnosno varijabilne primjene dušičnih gnojiva, ali koristeći samo nekoliko ranije utvrđenih valnih duljina odnosno multispektralnih kanala učinkovitih za detekciju nedostatka dušika. No, i dalje predstavlja tek dodatni alat u procjeni agronomskih svojstava koji se uvijek potvrđuje određenim brojem standardnih laboratorijskih analiza. Podaci prikupljeni na razini poljskog pokusa imaju dodatnu vrijednost za istraživanje, testiranje, procjenu i donošenje odluka ukoliko se integriraju u regionalni, nacionalni, lokalni, te okvir samog poljoprivrednog gospodarstva. Terenska spektroskopija kao dijagnostički pristup može unaprijediti biljno-uzgojne zahvate kroz procjenu prostorne i vremenske varijabilnosti određenih agronomskih svojstava. Bitno je napomenuti da i dalje postoji pitanje razvoja generalnih spektralnih algoritama neovisnih o razvojnom stadiju usjeva, kao i odabira tehnike modeliranja s najboljom sposobnošću predikcije. Osim toga, istraživanja bi se trebala usmjeriti na detaljniju analizu velikog broja hiperspektralnih podataka kako bi se izvela informacija bitna za što precizniju kvantitativnu procjenu istraživanih parametara.

PREGLED ISTRAŽIVANJA

Proizvođači ratarskih kultura sve su više pod pritiskom povećanja i prinosa i profita bez obzira na mjere zaštite okoliša i visoke cijene mineralnih gnojiva. Uniformna primjena gnojiva isključuje činjenicu da su zalihe dušika u tlu, biljci, kao i reakcija biljke na gnojidbu prostorno varijabilni. Stoga je jedan od glavnih ciljeva poljoprivrednika detaljniji i češći uvid u status dušika (N) u biljci i pravovremena gnojidba u svrhu povećanja agronomske učinkovitosti gnojidbe N uz istodobno smanjenje gubitaka N u okoliš. Učinkovitost gnojidbe N manja je od 50% u svjetskoj proizvodnji ozime pšenice (Thomason i sur., 2000.). Ostatak N veže se u tlu, dok se veći dio gubi denitrifikacijom, volatizacijom, emisijom dušičnih oksida, ispiranjem nitrata, te površinskim odnošenjem (Raun i Johnson, 1999.). Višekratno prihranjivanje dušikom tijekom vegetacijskog razdoblja omogućuje prilagodbu količina gnojidbe razvoju usjeva pri čemu se može očekivati maksimalno iskorištenje hranjiva (Boman i sur., 1995.). Učinkovita dijagnoza i dinamična regulacija statusa dušika u biljci trebala bi se bazirati na “real-time” praćenju razvoja usjeva i sadržaja dušika u biljci (Feng i sur., 2008.). Spektralna refleksija vegetacije u korelaciji je s razvojnim karakteristikama, te prema tome, ima potencijala pružiti informaciju o statusu dušika u biljci (Raun i sur., 2008.; Gong i sur., 2003.; Thenkabail i sur., 2000.). Preliminarna istraživanja pokazala su da ovaj pristup rješava pitanja prostorne varijabilnosti tijekom vegetacijskog razdoblja na način da se primjena dušika uskladi sa sadržajem dušika u biljci (Shanahan i sur., 2008.). Usvajanje ove vrste informacijske tehnologije od strane poljoprivrednika intenzivirat će se ukoliko odgovarajući sustav potpora odlučivanju postane dostupan kroz modifikaciju prikupljenih i obrađenih podataka u konkretne odluke u okviru “site-specific” gospodarenja u poljoprivredi (McBratney i sur., 2005.; Pimstein i sur., 2009.). Spektralni otisci ili spektralni uzorci vegetacije temelje se na interakciji energije i tvari, a određeni su refleksijom ili apsorpcijom zračenja kao funkcijom valne duljine elektromagnetskog spektra. Optička svojstva lista u rasponu od 350 do 1050 nm sadrže informaciju o koncentracijama biljnih pigmenata i staničnoj strukturi lista (McCoy, 2005.). Klorofil snažno apsorbira energiju valnih duljina oko 450 nm i 670 nm (Lillesand i sur., 2004.). Najveća osjetljivost refleksije i apsorpcije na promjene u sadržaju klorofila nalaze se u zelenom dijelu spektra (530-590 nm) i crvenom rubu (oko 700 nm) (Hatfield i sur., 2008.). Nasuprot tome, i refleksija i transmisija obično su visoke u NIR području spektra (700-1300 nm) zbog slabe apsorpcije zračenja staničnih čestica ili pigmenata te raspršivanja energije od staničnih stijenki mezofila (Slaton i sur., 2001.; Pinter i sur., 2003.). Također, mnoge vrste stresa kod biljke utječu na NIR područje, te se senzori koji mjere taj raspon elektromagnetskog zračenja mogu koristiti za određivanje razine promjena na vegetaciji (Lillesand i sur., 2004.) pod utjecajem suše, manjka dušika, niskog pH tla, zadržavanja vode. Gausman i sur. (1971.) navode razloge utjecaja razvojnog stadija usjeva na refleksiju mladog biljnog tkiva koje sadrži manje zraka unutar mezofila u odnosu na starije listove koji pokazuju smanjenu refleksiju NIR područja spektra. Scotford i Miller (2004.) u svom istraživanju iznose da se vrijednosti vegetacijskog indeksa NDVI ("normalized difference vegetation index") ozime pšenice postupno povećavaju s vremenom sve do maksimuma tijekom klasanja, nakon čega se počinju smanjivati idući prema kraju vegetacije. Sadržaj klorofila u listu i njemu proporcionalan sadržaj dušika u listu indikatori su potreba usjeva za dušikom (Kastori, 2005.; Follet i sur., 1992.). Filella i sur. (1995.) prepoznali su daljinska istraživanja kao pouzdanu i jeftinu metodu praćenja dušika u biljci te izdvojili refleksiju valnih duljina od 430 nm, 550 nm, 680 nm, te područja crvenog ruba kao bitne pokazatelje statusa dušika u pšenici. Osim toga, u usporedbi s ručnim klorofilmetrima, terenska spektroskopija pruža vrlo detaljne i bogate informacije zahvaljujući velikom broju valnih duljina i visokoj spektralnoj rezoluciji (Blackmer i sur., 1994.). Metode hiperspektralnih mjerenja predstavljaju novu tehnologiju koja bi mogla riješiti današnje probleme intenzivne poljoprivrede, a koja je već dovela do značajnog napretka u biljno-uzgojnim zahvatima zadnjih 30-ak godina. Mnoga istraživanja su provedena u svrhu procjene stanja ishranjenosti biljke N putem *in-situ* mjerenja spektralne refleksije usjeva (Stone i sur., 1996.; Raun i sur., 2002.; Hinzman i sur., 1986.; Xue i sur., 2004.; Tarpley i sur., 2000.; Read i sur., 2002.). Doprinos pojedinaca u razvoju metoda daljinskih istraživanja doveo je do shvaćanja refleksije i emisije lista biljke kao odraza strukture i starosti lista, biljne vrste, oblika biljnog pokrova, te sadržaja hranjiva i vode (Hatfield i sur., 2008.). Istraživanja utjecaja različitih količina dušične gnojidbe na spektralnu refleksiju ozime pšenice objavljena su u radovima Ayala-Silva i Beyl (2005.), Feng i sur. (2008.), Filella i sur. (1995.), Flowers i sur. (2003.), Sembiring i sur. (1998.), Jensen i sur. (2007.) i Fouche i sur. (1999.). Poznavanje spektralnih obilježja refleksije lista doprinijelo je definiranju vegetacijskih indeksa (VI), koji povezujući različite kombinacije valnih duljina elektromagnetskog spektra sa svojstvima biljke, mogu modelima predikcije kvantificirati određene agronomske varijable, odrediti njihove prostorne i vremenske varijacije, te još detaljnije istražiti informacije koje pruža spektralni otisak lista. Najpoznatiji algoritmi široke primjene su već spomenuti NDVI (Rouse i sur., 1974.) i RVI (“ratio vegetation index”) (Jordan, 1969.) jer sadrže informacije o fiziološkim uvjetima i razini stresa biljke koji potencijalno mogu djelovati na prinos. Tarpley i sur. (2000.) navode da omjeri između crvenog ruba (700 ili 716 nm) i NIR područja (755–900 i 1000 nm) imaju najbolju korelaciju sa sadržajem N u usjevu pamuka. Osbourne i sur. (2002.) zaključuju da je NIR područje elektromagnetskog spektra bilo presudno za procjenu prinosa zrna pšenice, s tim da se refleksija određenih valnih duljina mijenja ovisno o razvojnom stadiju. Stone i sur. (1996.) sugeriraju da se sadržaj TN u pšenici može procijeniti spektralnim indeksom u kombinaciji valnih duljina od 671 i 780 nm. Prema Reyniers i sur. (2006), podaci dobiveni daljinskim istraživanjima integriraju utjecaje različitih vanjskih i unutarnjih čimbenika na razvoj usjeva, te prema tome, predstavljaju veliki potencijal za primjenu u prognoziranju prinosa. Primjena terenske spektroskopije u gospodarenju N može smanjiti količine primijenjenih gnojiva uz istovremeno zadržavanje ili povećanje prinosa ratarskih kultura (Ferrio i sur., 2005.; Serrano i sur., 2000.; Wang i sur., 2003.). Raun i sur. (2002.) utvrdili su da procjene potencijalnog prinosa ozime pšenice tijekom vegetacijskog razdoblja primjenom indeksa NDVI mogu pridonijeti optimizaciji prihranjivanja dušikom i povećati NUE za više od 15 % u odnosu na ujednačenu primjenu dušika. NDVI izmjeren u razvojnim fazama kasnog vlatanja, klasanja i cvjetanja korelirao je s prinosom ozime pšenice (Freeman i sur., 2003.). U okvirima precizne poljoprivrede, pouzdana informacija o sadržaju N u biljnom tkivu nužna je prilikom primjene tehnologije varijabilnih količina N gnojiva u prihranjivanju usjeva. Zhao D. i sur. (2005.) ustanovili su da se refleksija u vidljivom dijelu spektra (556 i 710 nm) značajno pojačala pri nižim razinama N gnojiva što je dovelo i do pomaka refleksije u blisko-infracrvenom području prema kraćim valnim duljinama. Prema navedenim zaključcima, mjerenja spektralne refleksije lista mogu pružiti brz i dovoljno pouzdan način procjene ishranjenosti biljke dušikom. Spektralnu refleksiju kao funkciju razvojne faze i sadržaja N u biljnom tkivu istraživali su i Graeff i Claupein (2003.) koji su utvrdili da su mjerenja metodom terenske spektroskopije vrlo pouzdano identificirala različite varijante N gnojidbe i različit sadržaj N u kukuruzu. Primjenom diskriminantne analize na temelju vrijednosti vegetacijskih indeksa za određene valne duljine, svaki se spektar može klasificirati prema pripadajućem statusu N čime se omogućuje potencijalna procjena sadržaja N u pšenici (Filella i sur., 1995.). Različite statističke metode analize hiperspektralnih podataka za prognozu određenih agronomskih varijabli uključuju empirijske modele odnosa između standardno izmjerenih varijabli i spektralnih podataka (Korelacijska analiza – CA, Analiza glavnih komponenata – PCA; Parcijalna regresija najmanjih kvadrata – PLSR; Stupnjevana multipla linearna regresija - SMLR) (Atzberger i sur., 2010.; Gislum i sur., 2004.; Moron i sur., 2007.), procjene primjenom neuronskih mreža (ANN) kao klasifikatora i prediktora (Uno i sur., 2005.; Sui i sur., 1998.; Tumbo i sur., 2002.), te diskriminantnu analizu (DA) i klaster analizu (CLA) za procjenu kategorijske pripadnosti (Strachan i sur., 2002.; Jensen i sur., 2007.; Karimi i sur., 2005.). Rezultati ovih modela razlikuju se prema mjerilu snimanja, vrsti vegetacije, spektralnim područjima snimanja te složenosti modela (Hatfield i sur., 2008.). Kaleita i sur. (2006.) u svom su istraživanju iznijeli zanimljive rezultate prema kojima su ANN i PLSR modeli imali sličnu sposobnost predikcije određenih svojstava u kukuruzu. Neuralne mreže koriste puno prilagodljiviji oblik nelinearne jednadžbe u odnosu na tradicionalne linearne i jednostavne nelinearne analize (Kimes i sur., 1998.). Razvoj preciznih i općih modela za praćenje i procjenu statusa N u biljci na temelju spektralnih podataka još uvijek je u tijeku kao aktualan problem. No, glavni izazov znanstvenika koji se bave metodama hiperspektralnog snimanja u poljoprivredi jest da u potpunosti shvate potencijal ove tehnologije kao izvora vrlo korisnih informacija za donošenje odluka u agronomiji i unaprjeđenje gospodarenja na razini farme (Hatfield i sur., 2008.).

Hipoteza ovog istraživanja temelji se na postojanju veza između hiperspektralnih značajki lista ozime pšenice te agronomskih varijabli - sadržaja dušika i klorofila u listu, dušika u zrnu ozime pšenice, prinosa te agronomske učinkovitosti gnojidbe dušikom - određenih standardnim laboratorijskim analizama i terenskim mjerenjima. Glavni cilj doktorskog rada bio je procjena primjenjivosti VNIR spektroskopije u definiranju statusa dušika u biljci tijekom vegetacije kao i parametara ozime pšenice određenih nakon žetve, te određivanju utjecaja različitih količina dušične gnojidbe na spektralnu refleksiju i agronomska svojstva. Istraživanje je usmjereno na izvedbu i interpretaciju predikcijskih i klasifikacijskih modela temeljenih na hiperspektralnim podacima.

MATERIJALI I METODE

Lokacija i eksperimentalni dizajn

Istraživanje je provedeno na stacionarnom poljskom pokusu u sklopu hidromelioriranog poljoprivrednog zemljišta kojeg koristi tvrtka Moslavka d.d. (Agrokor) u području zapadno-panonske podregije Hrvatske (45°33´ N, 16°31´ E). Tip tla je definiran kao drenirani pseudoglej, ravničarski, distrični. Osim fizikalnih značajki tla koje pogoduju stagnaciji vode u površinskom dijelu profila te niskog sadržaja organske tvari, glavni ograničavajući čimbenik prinosa jest niska vrijednost pH tla, koja je djelomično i rezultat dugogodišnje mineralne gnojidbe dušikom. Teren karakterizira ravan reljef s prosječnom nadmorskom visinom 97.2 m. Šire područje obuhvaća umjerena kontinentalna klima s prosječnom godišnjom temperaturom od 10.7°C te prosječnom godišnjom količinom oborina od 865 mm (1965. – 1990. godina). Obje godine istraživanja bile su toplije od višegodišnjeg prosjeka (2008.: 1.9°C i 2010.: 0.8°C) dok je godišnji hod oborina bio izuzetno varijabilan u odnosu na prosjek. Na temelju bilance vode u tlu, utvrđeno je da je 2008. godina bila je mnogo sušnija od referentnog razdoblja i 2010. godine, koja se pak smatra ekstremno vlažnom u odnosu na prosjek.

Ruralni krajobraz čine manja obiteljska gospodarstva dok hidromeliorirana tla s intenzivnom poljoprivredom dominiraju u sklopu većih poljoprivrednih površina smještenih izvan naplavnog područja Lonjskog polja. Pokusna površina koristi se u sklopu višegodišnjeg istraživanja utjecaja mineralne gnojidbe dušikom (N) na prinos važnijih ratarskih kultura, agronomsku učinkovitost gnojidbe te ispiranje nitrate, u okviru znanstvenog projekta "Gnojidba dušikom prihvatljiva za okoliš" (Voditelj projekta: Prof.dr.sc. Milan Mesić; 178-1780692-0695, MZOŠ). Pokus ukupne površine od oko 4 ha postavljen je prema shemi bloknog rasporeda s 9 varijanti mineralne gnojidbe u 4 ponavljanja. Veličina osnovne parcele za svaku varijantu je 30 x 130 m, uključujući međuprostore. Pokusne varijante uključuju:

I. Kontrola–negnojeno,

II. N_0PK,

III. N_{100}PK,

IV. N_{150}PK,

V. N_{200}PK,

VI. N_{250}PK,

VII. N_{250}PK + Fosfogips,

VIII. N_{250}PK + Dolomit,

IX. N_{300}PK (kg N ha^{-1}).

Istraživanje u okviru doktorske disertacije uključivalo je vegetacijske godine 2008. i 2010. s ozimom pšenicom (*Triticum aestivum* L.) kao test kulturom i standardnim agro-tehničkim mjerama gospodarenja. Za ozimu pšenicu od ukupne količine fosfora i kalija odnosno PK gnojiva, 2/3 primijenilo se pri osnovnoj obradi tla (25-30 cm dubine), a 1/3 zajedno s 30 % N neposredno prije sjetve. Ostali dušik koristio se za prihranjivanje u tri obroka primjenjujući kalcij-amonijev nitrat (KAN): I. 25 %, II. 25 % i III. 20 %. Prvo prihranjivanje provelo se početkom proljetnog busanja, a drugo i treće u fazi vlatanja. Gnojidba za ozimu pšenicu iznosila je 500 kg kompleksnog mineralnog gnojiva NPK 10-30-20, za varijantu s količinom N od 200 kg, kao i za sve varijante s višim količinama N. Odgovarajuća količina fosfora (150 kg P_2O_5) i kalija (102 kg K_2O) za varijantu bez N te za varijante sa 100 i 150 kg N aplicirala se s pojedinačnim gnojivima, tripleksom (334 kg) i 60% kalijevom soli (170 kg). Korekcija gnojidbe N do metodikom predviđenih vrijednosti vršila se pojedinačnim gnojivima (urea, KAN). Za sjetvu ozime pšenice koristile su se sorta *Fiesta* (2008. godina) u količini od 300 kg sjemena ha^{-1} te sorta *Lucija* (2010. godina) u količini od 280 kg sjemena ha^{-1}.

Biljni materijal

Istraživanje je provedeno u dvije razvojne faze ozime pšenice (vlatanje – F8 i klasanje – F10.5, Feekes` skala) te nakon žetve tijekom vegetacijskih godina 2008. (*Triticum aestivum*

L. – sorta *Fiesta*) i 2010. (*Triticum aestivum* L. – sorta "*Lucija*"). Ozima pšenica je pouzdan indikator simptoma dušičnog stresa izraženih u obliku žućenja listova, smanjenja lisne površine i intenziteta fotosinteze (Kastori i sur., 2005.). Svojstva ozime pšenice pod utjecajem su genetičkih čimbenika u interakciji s ekološkim uvjetima (Balogh i sur., 2006.). U okviru ovog istraživanja, okolišni stres je zajedno s ostalim faktorima koji djeluju u poljskim uvjetima postignut kroz eksperimentalni dizajn, odnosno dvije vegetacijske godine, dvije razvojne faze i različite količine gnojidbe dušikom. Uzorkovan je prvi list ispod klasa ili zastavica na kojem su izvršena terenska mjerenja i laboratorijske analize. Zastavica čini oko 75 % efektivne lisne površine koja pridonosi nalijevanju zrna (Beuerlein, 2001.). Usjev pšenice u razdoblju vlatanja vrlo je osjetljiv na nedostatak dušika, čime se postiže dobra osnova za klasifikaciju biljaka s različitim statusom dušika pomoću analize *in situ* izmjerenih hiperspektralnih podataka.

Uzorkovanje biljnog materijala, terenska mjerenja i laboratorijske analize

Uzorkovanje lista ozime pšenice i terenska mjerenja izvršena su za svaku vegetacijsku godinu i sortu tijekom dvije razvojne faze ozime pšenice, vlatanja i klasanja, te neposredno prije žetve u svrhu analize zrna. Shema uzorkovanja podrazumijevala je direktno točkasto prikupljanje uzoraka biljnog materijala sastavljenog od 10 zastavica prema mrežnom rasporedu sa svake od 36 pokusnih parcela na površini od $1m^2$ na osnovi prostorne varijabilnosti određene različitim razinama gnojidbe. Spektralni podaci uključivali su nedestruktivno mjerenje refleksije elektromagnetskog zračenja (faktor refleksije) s površine lista, dok su agronomske varijable potrebne za razvoj kalibracijskog modela činili sadržaj ukupnog dušika u listu i zrnu (TN %), indeks sadržaja klorofila u listu (CCI), prinos zrna i učinkovitost gnojidbe dušikom (NUE). Precizna pozicija svakog uzorkovanja zabilježena je GPS uređajem (± 4 m). Isti uzorci biljnog materijala korišteni su za standardne laboratorijske analize sadržaja ukupnog N u suhoj tvari lista. Neposredno prije žetve na svakoj pokusnoj parceli uzeti su uzorci ozime pšenice s površine od 1 m^2 određene istim mrežnim rasporedom s ciljem određivanja gustoće sklopa i sastavnica prinosa. Nakon žetve određen je prinos zrna ozime pšenice koji je standardiziran na jedinice dt ha^{-1} ukupne suhe tvari. Stacionarna nedestruktivna mjerenja hiperspektralne refleksije lista ozime pšenice u poljskim uvjetima provedena su terenskim spektroradiometrom FieldSpec®3 (ASD Inc., SAD) s rasponom valnih duljina elektromagnetskog spektra od 350 do 1050 nm, intervalom uzorkovanja od 1.4 nm i spektralnom rezolucijom od 3 nm, bilježeći simultano podatke za 700 valnih duljina. Snimanje je izvršeno na 10 zastavica uzorkovanih na površini od 1 m^2 koje su predstavljale pod-uzorak svake pojedine eksperimentalne parcele (ukupno 360 spektralnih refleksija lista po razvojnoj fazi). Prosjek 10 uzastopnih mjerenja spektralne refleksije činio je jedan podatak za isti uzorak. Uprosječivanje spektra reduciralo je šum spektralnog signala u danom rasponu valnih duljina. Indeks sadržaja klorofila (CCI) određen je također brzo i nedestruktivno *in situ* ručnim klorofilmetrom CCM-200 Chlorophyll Content Meter (ADCBioscientific Ltd., Engleska) na istih 10 zastavica po svakoj parceli. Očitanja apsorbiranog i reflektiranog zračenja u dvije valne duljine (crveno - 653 nm i infracrveno - 931 nm) izmjerena su na sredini zastavice, te analizirana kao prosjek 3 uzastopna mjerenja istog lista. Uzorci lista (prosjek od 10 zastavica) i zrna ozime pšenice uzorkovani s 1 m^2 po parceli analizirani su standardnim destruktivnim laboratorijskim analizama sadržaja ukupnog dušika (TN %) metodom suhog sagorijevanja na instrumentu CHNS Elemental Analyzer Vario Macro prema HRN ISO 13878:2004. Navedene laboratorijske analize korištene su kao referentna mjerenja za kalibraciju predikcijskih modela baziranih na spektralnim podacima kao i za korelacijske analize. Dva dodatna indikatora uključena u izradu modela izračunata su na temelju izmjerenih i analiziranih varijabli. Empirijski su iz refleksije lista izvedeni vegetacijski indeksi (VI). Na temelju korelacijskih koeficijenata između refleksije svake pojedine valne duljine i agronomskih varijabli, a uzimajući u obzir i spektralna načela te uobičajeno korištene indekse, odabrana su najznačajnija spektralna područja i valne duljine na temelju kojih su izračunati NDVI $[(R_{NIR}–R_{RED})/(R_{NIR}+R_{RED})]$ i RVI (R_{NIR}/R_{RED}) indeks (R-refleksija; NIR-blisko infracrveno područje spektra; RED-crveno područje spektra). Kao relativni indikator agronomske učinkovitosti odabrana je učinkovitost gnojidbe dušikom (NUE) izračunata na temelju jednadžbe: NUE = (N $iznesen_G$ – N $iznesen_K$) / količina N u gnojivu % (G-tretirana parcela; K-kontrola; N iznesen– prinos zrna x sadržaj ukupnog N u zrnu) (Raun i sur., 2002.).

Statistička obrada podataka

Analiza spektralnih podataka metodama multivarijatne analize provedena je u softverskom alatu za analizu spektralnih podataka Unscrambler 9.7 (CAMO Software AS., Norveška) i u statističkom paketu Statistica 8.0 (StatSoft, Inc., SAD). Spektralni podaci vizualno su interpretirani u aplikaciji ViewSpec Pro 4.07. (ASD, Inc., SAD), nakon čega su modificirani iz .asd oblika datoteke u .ASCII oblik pomoću softvera ENVI (Research Systems, Inc., SAD). Originalni spektralni podaci obrađeni su metodama predtretmana i transformacije pomoću različitih algoritama kako bi se izbjegli fenomeni "pomaka" ("shift") i „šuma" ("noise") kojima je otežano razlučivanje varijabilnosti u podacima. Korišteni su prva i druga derivacija refleksije te linearizacija originalnih spektralnih podataka pomoću Savitzky-Golay filtera i polinomijalne funkcije drugog reda za derivacije i izravnavanje podataka (le Maire i sur., 2004.). Za potrebe analiza regresije uklonjene su valne duljine do 400 nm i iza 1040 nm zbog slabog signala instrumenta. U svrhu smanjenja količine podataka i olakšavanja kompleksnih statističkih analiza odabrana je svaka treća valna duljina čineći ukupno 231 podatak kroz puni spektralni raspon snimljene refleksije i njenih različitih transformacija. Nezavisne varijable činili su spektralni podaci – refleksija, 1. i 2. derivacija refleksije, vegetacijski indeksi (VI) i glavne komponente spektralnih podataka (PC), dok su sadržaj ukupnog dušika u listu i zrnu ozime pšenice (TN), indeks sadržaja klorofila (CCI), prinos i učinkovitost gnojidbe dušikom (NUE) odabrani kao zavisne varijable. Analiza glavnih komponenata (PCA) korištena je za smanjenje dimenzije podataka i kolinearnosti. Multivarijatni linearni statistički modeli (SLR, MLR, PLSR), nelinearna analiza strukture podataka pomoću učećih algoritama (ANN) i klasifikacijske analize (DA, CLA, ANN) generirani su u svrhu procjene biokemijskih i agronomskih svojstava ozime pšenice i diskriminacije kategorija dušične gnojidbe na temelju prve derivacije spektralne refleksije u obliku PC i VI. Povezanost spektralnih podataka lista pšenice s vrijednostima izmjerenih varijabli ozime pšenice istraživala se i analizom korelacije (CA) kako bi se identificirale značajne valne duljine i izračunali VI. Kroz statističku analizu multiple linearne regresije prikazani su glavni odnosi između spektralnih podataka i parametara ozime pšenice u obje godine istraživanja. S obzirom na bolju izvedbu modela sa sortom "Lucija" (2010) u fazi vlatanja (F8) u odnosu na kasniju fenofazu i sortu “Fiesta” (2008) u obje faze, druga godina istraživanja odabrana je za daljnje modeliranje. Drugi razlog za detaljnu analizu samo jedne godine odnosno sorte bio je svesti dimenzije doktorskog rada na optimalan volumen kroz smanjenje broja prikazanih rezultata. Vizualna analiza spektralnih krivulja, CA i MLR provedene su za sve podatke, no samo su rezultati MLR prikazani u cijelosti. Između četiri varijable ozime pšenice, model prinosa zrna pokazao je najbolju izvedbu i u CA i MLR, te na osnovu toga bio odabran za daljnje regresijske (SLR – VIs, PLSR, ANN) i klasifikacijske modele (DA, CLA, ANN). Svaki od navedenih modela testiran je u svrhu procjene pouzdanosti i predikcijske sposobnosti na temelju određenog broja laboratorijski utvrđenih vrijednosti zavisnih varijabli koristeći „leave-*n*-out” ili potpunu unakrsnu validaciju (svaki pojedinačni uzorak korišten je za testiranje procjene modela na temelju svih ostalih uzoraka). U svrhu analize točnosti i izvedbe modela odabrani su slijedeći statistički parametri: korelacijski koeficijent (r), koeficijent determinacije (R^2), korjenovana srednja kvadratna pogreška (RMSE) te funkcija pogreške u obliku sume kvadrata (SOS) i unakrsne entropije (CE) za ANN učeće algoritme. Modeli su međusobno uspoređeni u svrhu definiranja modela procjene svojstava pšenice s najboljom predikcijskom i klasifikacijskom sposobnošću. Statistička analiza razlika u sadržaju TN u listu i zrnu ozime pšenice, CCI u listu, NUE, prinosu i vegetacijskim indeksima NDVI i RVI prema varijantama gnojidbe provedena je analizom varijance (ANOVA) u statističkom paketu SAS 9.1 (SAS Institute Inc., SAD). Provjera značajnosti za cjelokupnu statističku analizu izvršena je za razinu vjerojatnosti pogreške od $p < 0.05$. Duncan-ov post-hoc test korišten je ukoliko je F test bio značajan za razinu vjerojatnosti pogreške od $p \leq 0.05$.

REZULTATI, RASPRAVA I ZAKLJUČCI

Varijante dušične gnojidbe kao i značajna varijabilnost unutar pojedinog tretmana uzrokovana klimatskim prilikama, genetičkim svojstvima sorte, razvojnim karakteristikama, značajkama tla i rezidualnog dušika u tlu (Serrano i sur., 2000.), rezultirali su širokim rasponom vrijednosti biokemijskih i agronomskih varijabli ozime pšenice: sadržaj TN u listu od 0.73 % do 4.27 %; CCI od 3.0 do 50.0; sadržaj TN u zrnu od 1.89 % do 2.67 %; prinos od 0.9 dt ha^{-1} do 50.1 dt ha^{-1}; i NUE od 3.1 % do 57.4 %.

Uzimajući u obzir sve vanjske čimbenike, zabilježen je očekivani utjecaj dušične gnojidbe na varijable ozime pšenice, što je bilo od izuzetne važnosti za daljnju analizu spektralnih podataka i modeliranje. Analizom varijance (ANOVA) utvrđene su statistički značajne razlike između srednjih vrijednosti sadržaja TN u listu i zrnu, CCI, prinosa, NUE kao i spektralnih indikatora NDVI i RVI izmjerenih za dvije sorte/vegetacijske godine, dvije vegetacijske faze pod utjecajem različitih varijanti mineralne dušične gnojidbe. Sve istraživane varijable ovisile su o razinama dušične gnojidbe, dok su im se vrijednosti povećavale s rastućim dozama dušika (ANOVA, $p < 0.001$). Do istih zaključaka došli su Barraclough i sur. (2010.) te Lopez-Bellido i Lopez-Bellido (2001.) u svojim istraživanjima. Kroz analizu ukupnog utjecaja svih faktora na *in situ* i laboratorijski izmjerene varijable, utvrđena je statistički značajna interakcija gnojidba x razvojna faza ukazujući time na širok raspon reakcija određenih svojstava. Statistički značajne interakcije sorta/vegetacijska godina x razvojna faza i gnojidba x sorta/vegetacijska godina zabilježene su samo za sadržaj TN u listu ukazujući na ovisnost razvoja biljke o klimatskim prilikama i režimu vode u tlu, te na značajni utjecaj dušične gnojidbe na svojstva sorte. Prema rezultatima ANOVA-e za ukupni utjecaj, sorte su reagirale slično na različite razine gnojidbe dušikom u obje razvojne faze. Osim toga, nepostojanje statistički značajne interakcije gnojidba x sorta/vegetacijska godina za sadržaj TN u listu izmjeren tijekom vlatanja čvrsta je osnova za zaključak da se spektralna istraživanja ozime pšenice mogu provoditi tijekom faze vlatanja neovisno o sortnim razlikama. Na osnovi navedenih rezultata i vizualne evaluacije spektralnih krivulja može se zaključiti da su hiperspektralni podaci odrazili kompleksne informacije o razvoju ozime pšenice. Na temelju ANOVA-e i statistički značajnih razlika u agronomskim varijablama između pojedinih varijanti, prosječne spektralne krivulje lista s devet varijanti gnojidbe grupirane su u tri klase čije su se srednje vrijednosti međusobno statistički značajno razlikovale. Pretpostavljajući da je za statistički značajno različite varijante gnojidbe dušikom ovo indikacija induciranog dušičnog stresa, spektralni otisak lista je kasnije korišten u klasifikaciji razine gnojidbe odnosno biljaka s različitim statusom dušika. Sličan pristup klasifikaciji na temelju spektralnih podataka koristili su i Alchanatis i Schmilovitch (2005.). Varijante dušične gnojidbe najbolje su se razlikovale na temelju spektralnih podataka lista snimljenih u fazi vlatanja sorte *Lucija*. Refleksija se povećala u blisko-infracrvenom području spektra (NIR) (> 740 nm), a smanjila u crvenom rasponu (660 – 690 nm) za kategoriju N_{250} i N_{300} (kg N ha^{-1}). Iste rezultate u svom radu potvrđuju Nguyen i Lee (2006.). Nedostatak dušika u kategoriji s Kontrolom i varijantom N_0 utjecao je na znatno povećanje refleksije crvenog dijela spektra te smanjenje NIR refleksije. Upravo ova promjena spektralne refleksije smatra se ključnom u detektiranju nedovoljne ishranjenosti biljke dušikom (Serrano i sur., 2000.). Najveće razlike u spektralnoj refleksiji između varijanti dušične gnojidbe kao i najjače korelacije s varijablama ozime pšenice utvrđene su u vidljivom i rubnom crvenom području spektra koje je najviše doprinijelo i ekstrakciji glavnih komponenata (PC). Kod refleksije uzoraka bez dušične gnojidbe uočen je pomak crvenog ruba prema kraćim valnim duljinama što navode i Penuelas i sur. (1994.) te Zhao i sur. (2005.). Veće količine apliciranog dušika te puni razvoj usjeva tijekom vlatanja (F8) utjecali su na smanjenje refleksije vidljivog i povećanje refleksije NIR spektra. Potpuno suprotan efekt u oba dijela spektra bio je rezultat niskog sadržaja klorofila u listu i ranijeg sazrijevanja na parcelama s nedostatkom dušika kao i tijekom faze klasanja (F10.5) ozime pšenice. Apsorpcija zračenja izmjerena u klasanju 2010. godine jasno je razlučila devet varijanti dušične gnojidbe uzimajući u obzir raspone vrijednosti agronomskih varijabli prema tretmanima. Tijekom vlatanja, varijante bez dušika spektralno su se izdvajale od ostalih koje su zbog intenzivnog rasta biljke i većeg usvajanja dušika imale i viši relativni sadržaj klorofila u listu. Usporedba varijante IX (N_{300}) između sorata/vegetacijskih godina ukazuje na jasniju diskriminaciju razvojnih faza pšenice tijekom 2008. godine što se objašnjava nepovoljnim klimatskim prilikama u obliku suše tijekom vlatanja koja se kasnije odrazila na slabijem razvoju i bržem sazrijevanju usjeva. Prateći ciljeve ovog istraživanja, može se zaključiti kako su različite količine dušične gnojidbe izravno utjecale na cjelokupnu razinu i raspon spektralne refleksije lista ozime pšenice. Slične rezultate navode i Hansen i Schjoerring (2003.). Visoke korelacije između spektralnih, biokemijskih i agronomskih varijabli (F8 – *Lucija*) dokazale su kako prinos ozime pšenice [r (NDVI : prinos) = 0.905] kao konkretan parametar može biti odabran za ulaznu zavisnu varijablu u prognostičkim modelima. Tijekom vlatanja utvrđena je i statistički značajna korelacija između CCI mjerenja i sadržaja TN u suhoj tvari lista (r = 0.922) što su već mnogo ranije potvrdili Fox i sur.

(1994.) te Evans (1983.). Dosadašnji rezultati ovog istraživanja upućuju na odabir faze vlatanja za prognozu prinosa ozime pšenice čime se pruža mogućnost za optimizaciju prihrane dušikom. Međutim, treba napomenuti da bi se daljnja istraživanja trebala usredotočiti na prognozu prinosa na temelju spektralnih snimanja ozime pšenice u ranijim fazama razvoja kao što je busanje, budući da je u našim uvjetima zbog kraće vegetacije naglasak na „ranoproljetnoj“ gnojidbi pšenice. Prema rezultatima korelacijskih analiza za svaku pojedinačnu valnu duljinu, jaka povezanost između spektralnih i agronomskih varijabli utvrđena je tijekom faze vlatanja. Abdel-Rahman i sur. (2010.), Penuelas i sur. (1994.), Read i sur. (2002.) te Zhao i sur. (2003.) u svojim istraživanjima ukazali su na slična spektralna područja značajna za predviđanje statusa dušika u usjevu. Većina značajnih valnih duljina nalazi se u vidljivom i rubnom crvenom prema NIR području spektra ukazujući na optička svojstva klorofila. Korelacijska analiza prve derivacije refleksije pokazuje maksimalni *r* sa sadržajem TN u listu na 602 nm i 671 nm (r = 0.83), s relativnim sadržajem klorofila (CCI) na 602 nm (r = 0.86), sa sadržajem TN u zrnu na 896 nm (r = -0.89), te s prinosom na 587 nm (r = 0.93). Wei i sur. (2008.) također utvrđuju blisku povezanost akumulacije dušika u listu ozime pšenice s položajem crvenog ruba. Sadržaj TN u listu i zrnu, CCI i prinos ozime pšenice pokazali su vrlo sličan uzorak korelacije sa spektralnim podacima što je opet povezano i s visokim korelacijskim koeficijentima između te četiri varijable. Prva derivacija refleksije rezultirala je s višim *r* vrijednostima u odnosu na originalne podatke. Najviši korelacijski koeficijenti između prinosa ozime pšenice i vrijednosti refleksije u crvenom do rubnom crvenom i NIR području spektra izdvojili su valne duljine za izračun vegetacijskih indeksa. Prema Jordan (1969.) i Rouse i sur. (1974.), izračunati su NDVI i RVI indeksi iz vrijednosti refleksije na 704 nm (λ_{CRVENO}) i 785 nm (λ_{NIR}). VI sa sličnim spektralnim značajkama utvrđeni su i u istraživanjima autora Carter (1994.), Barnes i sur. (2000.), Moges i sur. (2005.) i Stone i sur. (1996.) koji su utvrdili jake korelacije sa sadržajem klorofila, statusom dušika i prinosom zrna. Na osnovu provedenih korelacija generirani su jednostavni linearni regresijski modeli za procjenu prinosa ozime pšenice iz vrijednosti NDVI i RVI integriranih na temelju mjerenja u vlatanju (Benedetti i Rossini, 1993.). Utvrđene su vrlo jake korelacije između prinosa i vegetacijskih indeksa: r (NDVI) = 0.91 i r(RVI) = 0.87 (F8

- 2010. - sorta *Lucija*) ($p < 0.05$). Postignuti rezultati slažu se sa zaključcima skupine autora Girma i sur. (2006.) koji iznose da rezultati i korelacijskih i regresijskih analiza sugeriraju NDVI izmjeren sredinom vegetacijskog razdoblja kao pouzdan prediktor prinosa ozime pšenice. Međutim, u fazi klasanja iste sorte utvrđena je tek srednja korelacija između prinosa i istih vegetacijskih indeksa ($r \sim 0.5$). Statističke i ANN metode istraživanja spektralnih podataka korištene su za razvijanje modela predviđanja biokemijskih i agronomskih varijabli ozime pšenice iz spektralnih podataka u obliku prve derivacije refleksije. Ustanovljene su jednadžbe regresije između spektralnih varijabli u obliku glavnih komponenata i sadržaja TN u listu i zrnu, CCI, prinosa i NUE. Primjena prve derivacije refleksije kao nezavisnog prediktora pojačala je povezanost s varijablama ozime pšenice u odnosu na originalnu refleksiju i njenu drugu derivaciju (Zhao i sur., 2005.). Prva glavna komponenta (PC1) objasnila je 63 % varijabilnosti spektra lista, dok se 12 % varijabilnosti nalazilo u PC2. Multipla linearna regresija (MLR) rezultirala je spektralnim modelom od 7 PC koji je objasnio 83 % varijabilnosti u prinosu ozime pšenice te ustanovio visok udio varijance u biokemijskim svojstvima lista i zrna ($R^2 \sim 0.8$, $P < 0.05$) (F8, sorta *Lucija*). Modeli predviđanja varijabli sorte *Lucija* (2010.) razvijeni na temelju mjerenja u klasanju (F10.5) bili su manje precizni u usporedbi s vlatanjem (F8) što se prema nekim rezultatima (Ferrio i sur., 2005.) može objasniti početkom translokacije dušika u zrno te starenjem listova pod utjecajem nepovoljnih abiotičkih čimbenika (nedostatak N, prekomjerno zadržavanje vode zbog obilnih oborina) što je ograničilo mogućnost spektralne refleksije lista da učinkovito prati promjene u produktivnosti ozime pšenice. Vrijednosti koeficijenta determinacije (R^2) za unakrsno validirane modele iznosile su 0.67 za sadržaj TN u listu, 0.61 za prinos, 0.56 za sadržaj TN u zrnu i 0.54 za CCI. Predviđanje NUE iz žetvenih podataka procijenjenih hiperspektralnim snimanjem uspješno je provedeno (*r* za izmjeren vs. predviđen NUE bio je 0.81, $p < 0.05$). Jačina ove povezanosti ohrabrujući je rezultat koji ukazuje na visoku predikcijsku sposobnost modela baziranih na hiperspektralnim mjerenjima u vlatanju u procjeni agronomskih varijabli ozime pšenice (Wright i sur., 2004.). ANN modeli pokazali su se najučinkovitijima u prepoznavanju kompleksne veze između prinosa ozime pšenice (*Lucija*) i spektralne refleksije lista (kalibrirani i validirani setovi podataka s koeficijentima korelacije r = 0.95 odnosno r =

0.92, RMSEC = 2.57 dt ha^{-1} odnosno RMSEP = 4.41 dt ha^{-1}) u odnosu na odgovarajuće SLR-VIs, MLR i PLSR modele, ukazujući time na dobru izvedbu učenja algoritama ANN-a što su u svom istraživanju dokazali i Chen i sur. (2007.). Utvrđene su male razlike između MLR i PLSR regresijskih parametara nebitne za ostvarenje prognoze prinosa i optimizaciju višekratne prihrane dušikom tijekom vegetacijskog razdoblja. Izvedba PLSR modela s 8 faktora rezultirala je s najvećom postojanošću u predikciji koja je vidljiva u maloj razlici između RMSEC (4.10 dt ha^{-1}) i RMSEP (4.61 dt ha^{-1}) uz visoku predikcijsku sposobnost (validacija R^2 = 0.84), te pokazala da je moguće predvidjeti prinos zrna iz *in-situ* izmjerenih hiperspektralnih refleksija lista. Područja EM spektra značajna za PLSR model prinosa ozime pšenice obuhvatila su vidljivi dio spektra od 550 nm do 670 nm i 690 nm do 710 nm s područjem crvenog ruba od 730 nm do 770 nm. Iste spektralne zone utvrđene su i u korelacijskim analizama. Kao što je vidljivo iz dobivenih rezultata te istraživanja skupine autora Ferrio i sur. (2005.) i Atzberger i sur. (2010.), model razvijen na temelju eksplanatorne PC analize i PLSR kalibracije unatoč svom empirijskom karakteru uspio je integrirati fiziološke karakteristike iz refleksije velikog broja valnih duljina kako bi uspješno procijenio prinos ozime pšenice. Najviša pogreška predikcije utvrđena je za SLR model odnosa indeksa RVI i prinosa (RMSEP = 5.75 dt ha^{-1}). Točnost modela baziranih na vegetacijskim indeksima manja je za oko 23 % (validacija) u usporedbi s ANN procjenama, te 20 % u odnosu na PLSR model. Bez obzira na navedene razlike, NDVI i RVI ostvarili su vrlo jaku povezanost s prinosom ozime pšenice što pokazuju i rezultati unakrsne validacije modela: R^2 = 0.80 odnosno R^2 = 0.73. Pogreške predikcije (RMSEP) dobivene statističkim i ANN modelima mogu se smatrati vrlo prihvatljivima ukoliko se uzme u obzir složenost procjene prinosa i agronomskih varijabli pšenice na temelju spektralnih podataka (Uno i sur., 2005.). Na osnovi podjele varijanata dušične gnojidbe u tri statistički značajno različite kategorije (analiza varijance varijabli ozime pšenice), izvršena je klasifikacija spektralnih podataka izmjerenih u vlatanju sorte *Lucija* (2010.) prema principu koji su upotrijebili i Alchanatis i Schmilovitch (2005.). Klasifikacijskom analizom (DA, CLA i ANN) utvrđeno je da su hiperspektralna mjerenja tijekom najbržeg razvoja pšenice (F8) učinkovitija na mjestima s nedostatkom dušika nego tamo gdje postoji višak dušika što su zaključili i Jensen i sur. (2007.). Upravo je taj rezultat od izuzetne važnosti u preciznoj poljoprivredi u smislu prilagodbe prihrane dušikom zahtjevima biljke, identifikacije prostornih uzoraka fiziološkog stresa vegetacije te prognoze prinosa (Strachan i sur., 2002.). Klaster analiza pokazala je da se varijante bez dodanog dušika mogu razlikovati od ostalih kategorija, i to najviše na temelju plavog, zelenog i rubnog crvenog područja EM spektra koji ukazuje na promjene u sadržaju klorofila. Multivarijatna diskriminantna i klaster analiza bazirana na glavnim komponentama spektra (PC) klasificirale su mjerenja lista ozime pšenice statistički različitih kategorija statusa dušika sa 100 %-tnom točnošću. Karimi i sur. (2005) objavili su slične rezultate prema kojima je točnost klasifikacije dušične gnojidbe u kukuruzu na temelju spektralnih podataka iznosila više od 95 %. Jednako uspješna bila je i ANN klasifikacijska analiza. Neovisno o inventarizacijskom karakteru metoda klasifikacije korištenih u ovom istraživanju, ekstremne kategorije dušične gnojidbe bile su u potpunosti izdvojive [I (Kontrola, N_0) and III (N_{250}, N_{250} + dodaci, N_{300})], dok je manji udio varijabilnosti, utvrđen u srednjoj kategoriji s najoptimalnijim količinama dušika [II (N_{100}, N_{150}, N_{200})] (kg N ha^{-1}), vjerojatno posljedica utjecaja nekih dodatnih ekoloških faktora kao i prirodne varijabilnosti tijekom vegetacijskog razdoblja. U nastavku istraživanja, rezultate klasifikacijskih analiza potrebno je validirati pomoću novih spektralnih mjerenja kako bi bilo moguće pouzdano predvidjeti fiziološko stanje ozime pšenice. Rezultati ovog doktorskog rada potvrđuju visoki potencijal i primjenjivost terenske spektroskopije u procjeni stanja ozime pšenice tijekom razvoja i žetve jer se mogu koristiti u različitim prostornim mjerilima, širokom rasponu stresa dušika kao i u područjima s neograničavajućim količinama biljci raspoloživog dušika. Ključna spektralna obilježja i dobiveni algoritmi predstavljaju kalibracijsku osnovu za daljnje nedestruktivno i „real-time" praćenje statusa dušika u ozimoj pšenici hiperspektralnim daljinskim istraživanjima. Slijedeća nastojanja trebala bi se usmjeriti povećanju količine i složenosti uzoraka kako bi izvedeni model bio primjenjiv u raznovrsnim agroekološkim uvjetima. Istraživanje bi trebalo nastaviti u smjeru integracije zračnih i satelitskih hiperspektralnih informacija s modelima dobivenim mjerenjima u okviru ovog rada, te izrade softverske platforme s integriranim algoritmima za tehnologiju varijabilne primjene dušika (VRT). Rezultati prostorne raspodjele istraživanih varijabli ozime pšenice mogu se iskoristiti za promjene i poboljšanja agro-tehničkih mjera u gospodarenju narednim kulturama u plodoredu.

CONTENTS

1 INTRODUCTION

Efficient nitrogen (N) fertilization is crucial for economic cereal crop production and environmental protection. Understanding processes that govern N uptake and its distribution in wheat crops is a major importance with respect to both environmental concerns and quality of crop products. Because cca. one third of applied nutrients are used by wheat plants during vegetation period, it is very important to guide field management practices toward optimizing quantity of fertilizers, decreasing expenses of production and improving efficiency of wheat plants to absorb, accumulate and reutilize nitrogen. Nitrogen status in wheat leaves and grain represents indicator of N accumulation in plant indicating root system activity and translocation of nitrogen to the top of the plant. Wheat properties are mainly caused by effect of genetic factors in interaction with environment (Balogh et al., 2006) as a set of very variable abiotic factors which dynamic balance is essential for plant development through the whole vegetation period. Loss of chlorophyll (Chl), which is approximately proportional to leaf nitrogen content, is associated to environmental stress (Hendry and Price, 1993). This investigation aims to assess winter wheat nitrogen status influenced by different mineral nitrogen fertilization levels using Chl and N indirect detection. Location and the condition of the experiment during two vegetation periods (2008, 2010) are shown on Figure 1.1 and Figure 1.2.

Figure 1.1 - Satellite image of field experiment, *(Source: Google Earth, 2010).*

Figure 1.2 – Field experiment during stem extension, heading and harvest of winter wheat (2008, 2010).

However, to improve nitrogen use efficiency (NUE) in winter wheat, there is an increased need for reliable information on seasonal changes in crop N utilization. Both farmers and industry stand to realize better returns on their investment with analysis of crops prior to harvest. Better determination and classification of crop quality allow farmers to achieve higher profits, and industrial buyers can lower the risk of purchasing out-of-spec product (ASD Inc.).

New technologies using electromagnetic radiation reflected from plant leaves or canopies have potential in evaluating and determining plant nitrogen stress under field conditions. Ground-based remote sensing platform named hyperspectral field spectroscopy using many very narrow bands of visible and near infrared (VNIR) part of electromagnetic spectra may form a useful component of precision farming technologies and site-specific-crop management programs in order to improve N management in today`s agroecosystems. The sensor approach monitors actual growth conditions over time. In the framework of this research, field hyperspectral sensing was used for biochemical modeling based on quantitative calibration techniques and statistics in order to evaluate predictive ability of VNIR spectroscopy in identification of wheat N status and prediction of yield and NUE.

Implementation of airborne, satellite and proximal remote sensing in agronomy has made an exceptional progress in developed countries in the last few decades. Non-contact sensing or proximal remote sensing using field spectroradiometer has shown to be a good approach in detecting crop nutrient variability based on the relationship between the foliar optical properties, particularly specific chlorophyll and nitrogen spectral response, and its biophysical character. Some research has already proven that variable levels of chlorophyll/nitrogen concentrations in the leaves can be a good indirect estimator, obtained through spectral measurements, of a crop physiological condition. Vegetation has a unique spectral signature which enables it to be distinguished readily from other types of land cover or between itself by different species. The reflectance is low in both the blue and red regions of the spectrum, due to absorption by chlorophyll for photosynthesis. It has a peak at the green region which gives rise to the green color of vegetation. Chlorophyll has a relative low reflectance in the red part of the electromagnetic spectrum, which means high absorption. Low N content, dehydration or senescence affect leaf and stem chlorophyll content which visible reflectance is increased. On the other hand, healthy vegetation has a higher and brighter response in the NIR than in the green part of the spectrum. The changes in spectral response pattern of plant material result from phenologic changes through the season.

According to these considerations, field spectroscopy has a potential to be a highly sensitive technique that allows rapid leaf reflectance detection as an early pre-visual indicator of nutrient stress. Commercial applications have been developed where reflectance data are used as a basis for variable N rate application, but utilizing only few spectral bands already known to be efficient for detecting N stress.

Ground-based VNIR hyperspectral sensing, characterizing crop optical properties on the leaf level, can provide functional data and non-destructive, cost-effective, rapid and continuous quantitative assessment of crop variables important for optimizing N applications, thus replacing expensive and labor intensive laboratory testing used for traditional soil and plant tissue analysis, and eliminating the need for extensive field sampling. However, it is an additional tool in assessing specific agronomic variables in large-scale environments that should be always validated by specific number of standard laboratory analyses. Calibration samples from target sites are used for calibrating the hyperspectral sensor and developing calibration model. Inversion of the model is used for measuring reflected energy and predicting the condition of plant material. Defining specific spectral bands is also useful when deciding how spectral classes would be grouped into information classes when identifying nitrogen stress.

Ground-based remote sensing can be used to find optimal N rates directly or indirectly through estimation of the crop N variability. However, to be able to apply the required rate of N, the calibration of the data with the crop characteristics is critical.

Data collected for use at the subfield and field level have additional value for research, testing, evaluation and recommendation when incorporated in regional, national and also a farm framework. Coupled with GPS, field spectroscopy as diagnostic approach offers potential for improving farm management practices by assessing field spatial and temporal variability. High resolution field spectra characterizing crop optical features on the leaf and plant canopy level can provide, at a relatively low cost, a detailed but not necessarily precise quantitative assessment of crop growth and crop development variables (N status, Chl content, biomass, and yield). Much detailed analysis of narrow-band leaf spectra should be enabled using vegetation indices described as mathematical combinations of specific wavelengths values. Due to efforts for improving N use efficiency in winter wheat production and environmental protection, there is a high interest to explore and introduce in-field hyperspectral measurements as a source of useful information in monitoring crop growth that can be easily and effectively used for N management decisions. Several studies have illustrated the efficiency of this approach, showing overall yield increases and more uniform yield distributions. But, there is still an issue on using uniform spectral algorithms independent of the crop growth stage, and on selection of statistical modeling technique with best predictive ability. More efforts should be directed on much detailed analysis of narrow hyperspectral data to extract information which will gave more precise quantitative assessment.

This study addresses the potential of using non-contact measurements for discrimination of nitrogen status in winter wheat treated with different amounts of mineral nitrogen fertilizer by developing nitrogen calibrations using spectra of whole fresh leaves and, thus, is focused on the performance and interpretation of hyperspectral modeling techniques.

The most valuable and applicable scientific contributions of this doctoral research are as following:

- Discriminating N status in winter wheat using field spectroscopy will make contribution to world knowledge about hyperspectral characteristics of winter wheat
- Information established at the field level could be extended to regional level by satellite remote sensing technology
- The identified N-specific spectral algorithms may be used for image interpretation and diagnosis of wheat N status for site-specific N management
- Spectral data can be used for identifying areas where there is potential of crops to respond to additional N
- Estimation of NUE and yield based on spectral data will contribute to winter wheat non-destructive and real-time monitoring and may play a fundamental role in supporting policy formulation and decision making in N fertilizer management in wheat production

2 BACKGROUND

Crop nitrogen status is known to be a key indicator and one of the most critical and variable factors for evaluating crop growth, increasing yield and improving grain quality (Wei et al., 2008). Nitrogen application is usually the most important input for winter wheat production because of yield increases associated with increasing levels of nitrogen (N) fertilizer (Varga et al., 2001). However, cereal producers are today under pressure to increase yields and maintain profitability against a background of environmental constraints and high N fertilizer costs. Uniform applications within fields discount the fact that N supplies from the soil, crop N uptake, and crop response are spatially variable. One of the main objectives of agricultural producers is to accurately detect plant N status and provide N fertilizer in a right time to improve crop yield and quality, increase N use efficiency, optimize farm profitability, and to minimize N losses to the environment. Current N management strategies for world cereal production systems have resulted in low NUE, averaging only around 33% of fertilized N recovered. Of the more than 56 million tones of fertilizer N applied to all cereal crops each year, 66 % is not recovered by the crop, some of which is immobilized in the soil and the rest is lost by denitrification, volatilization, gaseous plant N loss, leaching and surface runoff (Raun and Johnson, 1999). The higher N rates generally result in decreased NUE values, reflecting a poor crop use of fertilizer, as investigated for winter wheat (Sieling et al., 1998; Arnall et al., 2009; Vuković et al., 2008; Lopez-Bellido and Lopez-Bellido, 2001). An increase in NUE of 20% would result in a savings in excess of $4.7 billion per year (Raun and Johnson, 1999). Nitrogen use efficiency (NUE) is known to be less than 50% in winter wheat grain production systems (Thomason et al., 2000). Current methods of determining N fertilization rates in winter wheat are based on farmer projected yield goals and fixed N removal rates per unit of grain produced (Lukina et al., 2001). A mid-season split application of N fertilizer provides room for adjusting rates according to crop growth thus maximum utilization of fertilizer is expected (Boman et al., 1995). This N management strategy can be further improved by applying topdressing N rates based on winter wheat needs to maximize yield and minimize input costs (Arnall et al., 2009). Development of innovative strategies that improve NUE and minimize off-field losses is crucial to sustaining cereal-based farming. Effective diagnosis and dynamic regulation of plant N status must be based on real-time monitoring of growth characters and nitrogen levels in crop plants (Feng et al., 2008). Non-destructive, indirect measures of crop health have been identified as possible alternatives to soil N testing for evaluating crop growth and production capability and making management decisions about agricultural inputs (Moges et al., 2004). Remote sensing (RS), especially ground based hyperspectral reflectance measurements in the visible and near infrared part of electromagnetic spectrum based on leaf optical properties have been evolved as potentially valuable agronomic tool in site-specific management, aimed to solve problems facing intensive agriculture. Crop spectral reflectance is well correlated with crop growth, so has the potential to provide information about N status (Raun et al., 2008). The main task of agronomic RS is to determine the sensitive bands of spectral reflection and their derived parameters characterizing vegetation canopies for indicating growth status, and then to determine the quantitative relationships between spectral properties and agronomic parameters (Feng et al., 2008). Preliminary research shows that this approach addresses the issue of spatial variability and is accomplished at a time within the growing season so that N inputs are synchronized to match crop N uptake (Shanahan et al., 2008). Remotely sensed estimates of crop condition during the growing season as early warning of yield reducing stress are potentially important source of spatial and temporal data for precision agriculture and a valuable input to crop growth and yield models. Within-field variation in red and NIR canopy reflectance, and hence in normalized difference vegetation index (NDVI) which uses these parts of the spectrum in the algorithm, may result from spatial patterns in N deficiency (Eitel et al., 2008). RS coupled with global positioning system (GPS) and geographical information system (GIS) represents spatial information technology which concept is to sustainable manage agricultural systems based on information and knowledge. That means to use these comprehensive data for applying variable-rate technology (VRT). Optical sensing equipment that employs this approach is now commercially available to growers and fertilizer dealers. But, adoption of this information technology by farmers is expected to increase as appropriate decision support systems become available to translate the acquired data into site-specific management decisions (McBratney et al., 2005; Pimstein et al., 2009).

The foundations and main concept of remote sensing relies on fact that information about an object can be obtain through the analysis of data acquired by a device that is not in contact with the object under investigation (Lillesand et al., 2004). Corresponding with this explanation, field spectroscopy as proximal type of RS is the measurement of the interactions of radiant energy with *in situ* objects in the environment (McCoy, 2005). Remote sensing has its physical principle in the theory of electromagnetic spectrum and reflectance of particular wavelengths of this spectrum (Havrankova, 2007). Spectral signatures or spectral response patterns of plant material are based on energy-matter interaction and defined by their reflectance or absorbance, as a function of wavelength in the electromagnetic spectrum. Hyperspectral sensing using specific narrow bands and their mathematical combinations provides critical information in quantifying crop variables compared to broad bands (Gong et al., 2003; Thenkabail et al., 2000). Under controlled conditions, the signatures result from electronic transitions of atoms and vibrational stretching and bending of structural groups of atoms that form molecules. Fundamental features in reflectance spectra occur at energy levels that allow molecules to rise to higher vibrational states (Shepherd and Walsh, 2002). Spectral properties of vegetation are influenced by a limited set of spectrally active compounds (Figure 2.1).

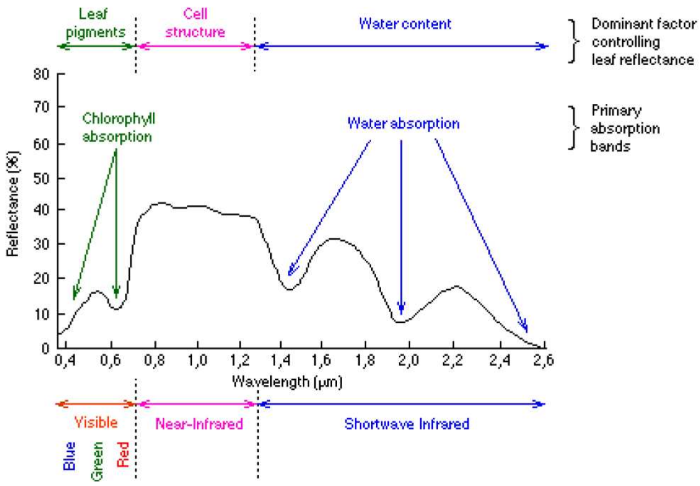


Figure 2.1 – Typical spectral response characteristics of green vegetation (from Christensen, 2004).

Reflectance in the VNIR region (0.4-1.0 μm) is dominated by chlorophyll pigment, and reflectance in the SWIR (short-wave infrared) region (1.0-2.5 μm) contains overtones for C-H, N-H, C-O, and CH_2 bearing compounds (protein, lignin, sugar and cellulose) (Curtiss and Goetz, 1994). Leaf optical properties, gathered in the 350-1050 nm region of the electromagnetic spectrum, contain information on plant pigment concentrations and leaf cellular structure. “Peak-and-valley” configuration of spectral reflectance curves for healthy green vegetation is consisted of reflection and absorption features. Interaction of electromagnetic energy with leaf pigments is restricted to the visible wavelengths (400-700 nm) (McCoy, 2005). Chlorophyll (Chl) strongly absorbs energy in the wavelength bands centered at about 450 and 670 nm (Lillesand et al., 2004). When Chl is abundant in the leaf, it dominates both reflection in the green and absorption in blue and red bands. If a plant is subject to some form of stress that interrupts its normal growth and productivity, it may decrease or cease Chl production. The result is less Chl absorption in the blue and red bands (Lillesand et al., 2004). The highest sensitivity of reflectance and absorption to Chl variation is in the green (530-590 nm) and in the red edge (around 700 nm) (Hatfield et al., 2008). In the visible wavelengths relatively little energy is transmitted through the leaves. Reduced leaf moisture results in an overall increase of reflectance in this part of the spectrum (McCoy, 2005). Figure 2.2 shows a depiction of the plant leaf structure and how different parts of the plant react with light energy. Chlorophyll is contained within numerous membranes called chloroplasts located in the stem and leaf structure, giving the plant its green color.

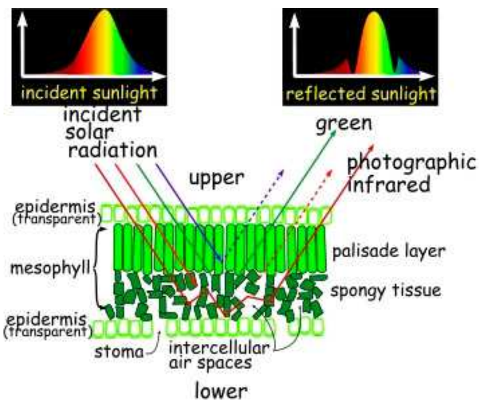


Figure 2.2 – Interaction of leaf structure and Sun energy represented with different parts of electromagnetic spectrum *(Source: leddv.com/Basic_FACTs_E_xp_15.html).*

By contrast, reflectance and transmittance are both usually high in the near-infrared regions (700 to 1300 nm) because there is very little absorbance by pigments and also because there is considerable scattering at mesophyll cell wall interfaces (Slaton et al., 2001; Pinter et al., 2003). In that region, a plant leaf typically reflects 40-50 % of the energy incident upon it. Most of the remaining energy is transmitted, since absorption in this spectral region is less than 5 %. Plant reflectance in the range 700-1300 nm results primarily from the internal structure of plant leaves (Figure 2.3). Because this cell structure is highly variable between plant species, it provides discriminating between species, even if they look the same in visible wavelengths. Also, many plant stresses alter near-infrared region, and sensors operating in this range can be used for vegetation stress detection (Lillesand et al., 2004).

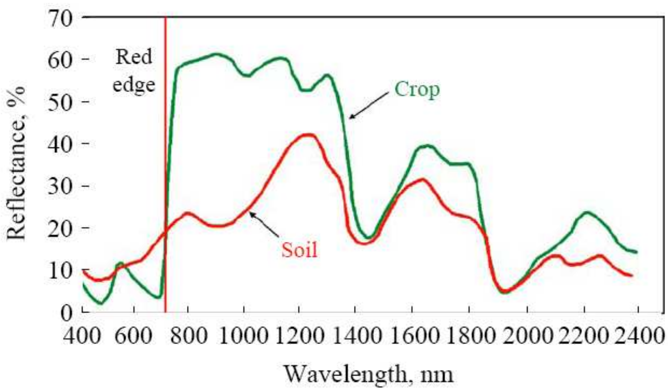


Figure 2.3 – Typical reflectance spectral signatures of soil and crop (after Scotford and Miller, 2005).

Gausman et al. (1971) reported the reason for the impact of growth stage on reflectance reading of young plant tissue which had less air space within the mesophyll than older leaves, which, thus, showed decreased NIR spectral radiance. For example, relationship between NDVI and N status in winter wheat suggests the need for growth stage specific calibration (Sembiring et al., 2000). Scotford and Miller (2004) found that NDVI values of winter wheat canopies gradually increased with time until a maximum (GS 45 - mid-booting) was reached before starting to decrease. This sharp dissimilarity in reflectance properties between visible and NIR wavelengths underpins a majority of remote approaches for monitoring and managing crop and natural vegetation communities (Knipling, 1970).

Leaf chlorophyll and N concentration in the leaf dry matter are indicators for crop nitrogen requirements (Kastori, 2005; Follet et al., 1992). Approximately 10% of the plant total nitrogen content is stored in chlorophyll molecules (Christensen, 2004) (Figure 2.4). Chlorophyll content is approximately proportional to leaf nitrogen content, so quantifying Chl content gives an indirect measure of nutrient status (Filella et al., 1995). The same authors recognized remote sensing as a reliable cost-effective method that could be used to monitor N status in crop. They reported that the use of reflectance at 430 nm, 550 nm, 680 nm, and red edge wavelengths offers potential for assessing N status of wheat (Figure 2.1). N status of wheat can be measured using tissue sampling or estimated using spectral reflectance devices (Wright et al., 2004; Osbourne et al., 2002). Compared to the hand-held chlorophyll meters, reflectance spectroscopy offers a wealth of information due to the large number of narrow wavebands. For in-season adjustments of N applications, spectral measurements seem to be the most efficient technique (Blackmer et al., 1994). Nitrogen applied before stem elongation in wheat can increase yield if the crop is deficient in N, while applications of N fertilizer after stem elongation increase protein content (Fisher et al., 1993). Still, the main issue relies in selecting more general spectral indicators or specific wavelengths, which will be able to quantify crop N status or potential yield regardless of growth stage and cultivar as well.

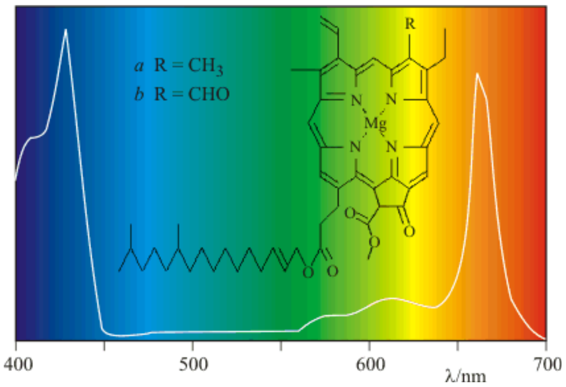


Figure 2.4 – Main absorption peaks of chlorophyll molecule *(Source: www.ktfsplit.hr/glossary/image/chlorophyll.gif).*

Spectral sensing has provided valuable insights into agronomic management over the past 30 years. Many studies have been conducted to assess leaf/canopy N status of crop, estimate yield and determine N input recommendations by in-field ground based measurements of crop spectral responses (Stone et al., 1996; Raun et al., 2002; Hinzman et al., 1986; Xue et al., 2004; Tarpley et al., 2000; Read et al., 2002). The contributions of individuals to remote sensing methods have lead to understanding of how leaf reflectance and leaf emittance changes in response to leaf thickness, species, canopy shape, leaf age, nutrient status, and water status (Hatfield et al., 2008). Spectral reflectance data from leaves and canopies have been related to specific biophysical and biochemical variables, which are important in modeling crop growth and nutritional status (Vogelmann, 1993). Most of the work in this field has encouraging results reached by many scientists. Studies on responses of plant spectra corresponding to different N fertilization levels were reported by Strachan et al. (2002), Alchanatis and Schmilovitch (2005), Clay et al. (2006), Zhao et al. (2003) for corn, Ayala-Silva and Beyl (2005), Feng et al. (2008), Filella et al. (1995), Serrano et al. (2000), Flowers et al. (2003), Sembiring et al. (1998), Jensen et al. (2007), Fouche et al. (1999) for wheat, Zhao et al. (2004), Buscaglia and Varco (2002) for cotton, Jongschaap and Booij (2004) for potato, Jensen et al. (1990) for barley, Zhang et al. (2006) for rice, Penuelas et al. (1994) for sunflower. All these studies have shown correlations between various parts of the reflected spectrum and plant N status. Classifying crops by nitrogen stress is of great interest in precision agriculture. Understanding of leaf reflectance has lead to various vegetative indices (VI) relating specific waveband combinations to plant properties with purpose to quantify various agronomic variables, detect their spatial and temporal variations to exploit the information content of detailed leaf spectra. These mathematical indices reduce complex spectra to a single value. VIs are based on knowledge of the reflectance properties of the biochemical components in leaves and can be targeted to estimate for example N content in leaf (Adams et al., 1999), and, therefore, can be useful for obtaining information about the physiological and stress conditions that could potentially affect crop yield. The Normalized Difference Vegetation Index (NDVI) (Rouse et al., 1974) and Simple Ratio Index or Ratio Vegetation Index (SRI and RVI, respectively) (Jordan, 1969) based on defined specific red and infrared reflectance bands are widely used algorithms for monitoring, analyzing, forecasting and mapping temporal and spatial distributions of physiological and biophysical characteristics of crops because they are more steady and reliable compared to single wavelength use. While some scientists report strong relationships using novel indices (Carter, 1994; Barnes et al., 2000), most applied research is still using common NDVI and SRI (Raun et al., 2001; Wright et al., 2004; Lukina et al., 2001; Moges et al., 2004; Sembiring et al., 2000; Reyniers et al., 2006; Zhang et al., 2006; Zhu et al., 2008). Defining VI that maximizes the sensitivity to the parameter of interest while minimizing the sensitivity to other internal and external variables and conditions has been the main issue of many studies (Broge and Leblanc, 2001; Haboudane et al., 2004; Muller et al., 2008; Thenkabail et al, 2002). Yao et al. (2010) designed a new method to extract hyperspectral information and identified novel sensitive bands for simple spectral indices (contour maps) to quantify the relationship of leaf N accumulation to spectral features and to establish applicable N monitoring model for modern winter wheat production. The advantages of these matrix plots made of regression coefficients between VIs and crop variables are that they give a quick overview of thousands of wavelength combinations and make it possible to detect wavelengths of interest for further analysis (Thenkabail et al., 2000). There are several studies where green and near infrared bands and their combinations were found most sensitive to Chl/N content variations in crop leaf and highly correlated with grain yield (Sembiring et al., 1998). Blackmer et al. (1994) found that linear relationship between leaf N content and leaf reflectance at 550 nm had a coefficient of determination of 0.90. Gitelson et al. (1996) substituted the green spectral waveband for the red spectral waveband in the NDVI and showed this index was more responsive to leaf chlorophyll content than the original NDVI. Strachan et al. (2002) noted a shift in importance from green-based derivatives to red-based derivatives from mid to late season and attributed to the natural reduction in green pigments as the crop entered senescence. These reports can be explained by work of Jacquemoud and Baret (1990) who found reflectance sensitivity to Chl content is greater at 675 nm than at other wavelengths for low content, and greater at 550 nm for medium-to-high content (after Inoue, 2003). Xue et al. (2004) reported that a robust model between green ratio vegetation index (GRVI) and rice leaf N accumulation was established independent of growth stages and N level. Tarpley et al. (2000) found that ratios between red-edge (700 or 716 nm) and near-infrared (755–900 and 1000 nm) provided the best correlation with leaf N concentrations in cotton, similar to results of Zhao et al. (2007) who reported the same relationships to leaf area index (LAI) and chlorophyll content density (CCD) in cotton. However, Fernandez et al. (1994) found that linear combination of green and red canopy reflectance can be related to nitrogen content of plants, but independently of plant treatment, similar to results of Osbourne et al. (2002) who, among that, concluded that NIR region was crucial for estimation of grain yield but with the particular wavelengths of importance changing with growth stage. Stone et al. (1996) suggested that total N concentration in wheat plant could be estimated with the spectral index based on the combination of two spectral bands at 671 and 780 nm. The identified N-specific spectral algorithms may be used for diagnosis of winter wheat N status for site-specific N management. But still, growth stage and N level independent spectral model for specific crop is missing. Numerous significant hyperspectral parameters are different between researchers and change with crop growth. Only specific spectral regions and wavelength ranges that mostly contribute, for example, to yield predictions can be established regardless of growth stage or cultivar. Yet, yield prediction model or N monitoring model if dependent of growth stage is still under influence of some other external factors when validated in other situations (unfavorable soil and climate conditions, plant diseases, other nutrients deficiencies). To solve that problem, in quantitative analysis of hyperspectral remote sensing it is necessary to provide diverse training samples, and increase the amount, complexity and representation of samples so that the derived model can be applied under varied conditions. Nevertheless, it is obvious that remote sensing data integrates manifestation of effects of different external and internal factors on the crop growth, and hence can provide immense potential for use in crop yield forecasting (Reyniers et al., 2006). Detection of N status in winter wheat and prediction of yield, yield variables and NUE from in season spectral reflectance measurements were the primary objectives of several studies. Their main conclusions follow the fact that hyperspectral sensing in site-specific N management can reduce N applications while maintaining or increasing crop yields (Ferrio et al., 2005; Freeman et al., 2003; Jensen et al., 2007; Benedetti and Rossini, 1993; Serrano et al., 2000; Munden et al., 1994; Wang et al., 2003). An indirect approach was proposed by Raun et al. (2002), who found midseason estimates (using NDVI measurements) of potential yield of winter wheat, would help growers to adjust midseason top-dress N application. They reported that N fertilization estimated on this way with respect to spatial scale increased NUE by more than 15 % when compared to traditional practices which applied N at uniform rates. Mullen et al., (2003) used spectral sensor measurements at different wheat growth stages to predict winter wheat yield response to N and to predict optimal N rate. Lukina et al. (2000) investigated influence of winter wheat production on plant spectral properties and reported that NDVI increased with N fertilizer rate at Feekes growth stage 4. Labus et al. (2002) found strong relationships between wheat yields and integrated NDVI over the entire growing season, and with late-season NDVI parameters on satellite level. Zhao C. et al. (2005) conducted research on winter wheat and found that vegetation index derived from the canopy spectral reflectance at green and red bands, was significantly correlated to the leaf N content at anthesis stage, and also highly significantly correlated to the final grain protein content. Li et al., (2008) found that RVI can be used to estimate nitrogen status for winter wheat in over-fertilized farmers' fields before heading using a handheld spectroradiometer. Although this excess N fertilization seldom occurs in agricultural production practice, future attention should be paid to explore additional hyperspectral indices applicable to a wider range of N levels in wheat plants. The NDVI at critical growth stages such as booting, heading and flowering has been correlated to final winter wheat yield (Freeman et al., 2003). But, very few papers reported achievement of robust models for crop N monitoring. NIR region was found to be very sensitive to plant physiological changes, so it is very questionable to use it for robust VIs. According to the recent literature, visible range is more stable than NIR in late-season predictions of leaf N status or yield. Xue et al. (2007) reported that the strong dependence of yield on crop N status may explain significant strong correlation between GRVI and winter wheat grain yield at mid-filling stage. In the context of precision farming, quantitative information on plant N concentration is necessary to apply variable rate technologies of top-dressing fertilization. Radiometric measurements are useful for monitoring crop conditions, especially N and chlorophyll (Chl) assessment (Stroppiana et al., 2008). They found the most suitable bands in the visible (blue/green) region of the electromagnetic spectrum where N/Chl compounds play a key role in radiation absorption. Hansen and Schjoerring (2003) reported that visible spectral range, mainly in the blue region, proved to be better to find linkage to Chl and N content in winter wheat leaf tissue using narrow band indices and partial least square regression (PLSR). Zhao D. et al. (2005) found that spectral reflectance in visible part of spectrum (556 and 710 nm) increased significantly as N fertilizer rate decreased which also caused a red-edge shift to shorter wavelength. Subsequently the reduction increases leaf reflection and transmission, decreases leaf absorbance, and shortens the red-edge position (REP), defined as the inflection point that occurs in the rapid transition between red and near-infrared (Scotford & Miller, 2005). At the transition from red to NIR wavelengths, leaf reflectance greatly increases. The positioning of red edge has been correlated mainly to chlorophyll content as well as plant phenological stages and plant stress (Jago et al., 1999; Filella and Penuelas, 1994). After Broge & Mortensen (2002) REP can be defined as the wavelength where the first derivative of the spectral reflectance is the maximum. Wei et al. (2008) found a very close relationship between leaf nitrogen accumulation in winter wheat and red edge position. Reflectance spectra as a function of growth stage and N status were investigated in study conducted by Graeff and Claupein (2003) who referred that reflectance measurement precisely reflected different N treatments and different N status of corn plants. By discriminant analysis based on the pigment indices of reflectance at specific wavelengths, each reflectance spectrum can be assigned to a different N status class which offers a potential for assessing N status of wheat (Filella et al., 1995). Assessment of N status in winter wheat from narrowband spectral reflectance indices was investigated to improve N management and NUE (Li et al., 2009; Raun et al., 2002; Reusch, S., 2005). Plants spectral signatures change according to the plant's physiological status and growth conditions (Card et al., 1988), hence the wheat temporal status and spatial variation over a field is essential information in reaching good fertilizer strategies.

Different statistical methods applied for remote sensing of agronomic parameters using agronomic information retrieval algorithms have been developed and confirmed by several studies as efficient in extracting and creating reliable models in agronomy. These approaches include empirical models between surface/destructive-measured crop parameters and spectral variables (Correlation analysis – CA, Principal component analysis – PCA; Partial least squares regression – PLSR; Stepwise multiple linear regression - SMLR) (Atzberger et al., 2010; Wold et al., 2001; Ferrio et al., 2005; Gislum et al., 2004;

Gong et al., 2003; Moron et al., 2007), estimations using an artificial neural network (ANN) as classifier and predictor (Uno et al., 2005; Sui et al., 1998; Tumbo et al., 2002; Liu et al., 2010), and discriminant analysis (DA) for prediction of group membership (Strachan et al., 2002; Jensen et al., 2007; Karimi et al., 2005; Penuelas et al., 1994; Zhao et al., 2005). Patterns of the data are modeled and calibrated to be applied to future data in order to predict the identity or condition of biophysical material. The results from these methods vary by scale of observation, number and diversity of samples, type of vegetation, spectral bands, spectra preprocessing and the sophistication of the models (Hatfield et al., 2008). Kaleita et al. (2006) reported interesting results where a novel range operator-enabled genetic algorithm (ROE-GA), designed to consider the shape of the spectra, had similar predictive capabilities to the ANN and PLS, to evaluate hyperspectral reflectance data for detecting onset of pollen shed in maize. Neural networks employ a more powerful and adaptive nonlinear equation form as compared to traditional linear and simple nonlinear analyses (Kimes et al., 1998). Yang et al. (2009) found generalized regression neural network (GRNN) model based on first derivative spectra to be the best model for the prediction of rice LAI and green leaf chlorophyll density (GLCD) which resulted from the strong capacity for nonlinear mapping and good robustness of GRNN. Research of Liu et al., (2010) verified that the back propagation neural-network model could provide an effective and faithful estimation of leaf chlorophyll variation based on the four performance spectral indices in rice stressed by heavy metals. Results of recent research are different, but generally the ANN and PLSR models seemed to have equally successful performance. The analysis approach is different and processing of spectral data and algorithms need to be quite simplified for common usage in agronomy.

Due to the fact that spectral parameters may be crop-growth stage-year-site specific, development of accurate and general models (growth stage or whole-season specific) to monitor and predict N status in crop plants from reflectance data is still an on-going task. The major challenge for remote sensing researchers is to fully realize the potential of hyperspectral data as a source of useful information that can be used for agronomic management decisions. Using field spectroscopy to help make agronomic decisions may provide a tool to improve field-scale management (Hatfield et al., 2008).

3 HYPOTHESIS AND OBJECTIVES OF RESEARCH

Hypothesis:

1. Different N fertilization treatments have effect on winter wheat yield, NUE, leaf TN content, CCI, grain TN content and spectral properties
2. Different N fertilization rates can change plant spectral response
3. Relationship between winter wheat leaf hyperspectral properties and biochemical variables measured by standard laboratory analysis exists
4. Models developed from in-season spectral data can accurately predict leaf TN content, CCI, grain TN content, yield and NUE and classify winter wheat samples according to different fertilization treatment using different algorithms

Objectives:

Evaluation of potential use and reliability of field reflectance spectroscopy for in-season spatial and temporal assessment of N status in winter wheat under different N fertilization levels with final purpose to improve farm N management through increasing NUE in wheat production:

1. To determine effect of different N fertilization levels on winter wheat yield, NUE, leaf TN content, CCI, grain TN content and spectral properties
2. To investigate if a N rates discrimination model could be established based on winter wheat spectral response regardless of growth stage
3. To identify significant wavelengths and their combinations as a potential indicators for estimating winter wheat variables under field conditions
4. To determine if it is possible to predict winter wheat biochemical variables from the hyperspectral data (reflectance values, spectral indices)
5. To establish a reliable model for winter wheat yield and NUE assessment based on hyperspectral data by comparing predictive capability of different analytical techniques and algorithms

6. To determine if it is possible to classify different N fertilization rates from analysis of leaf reflectance spectra

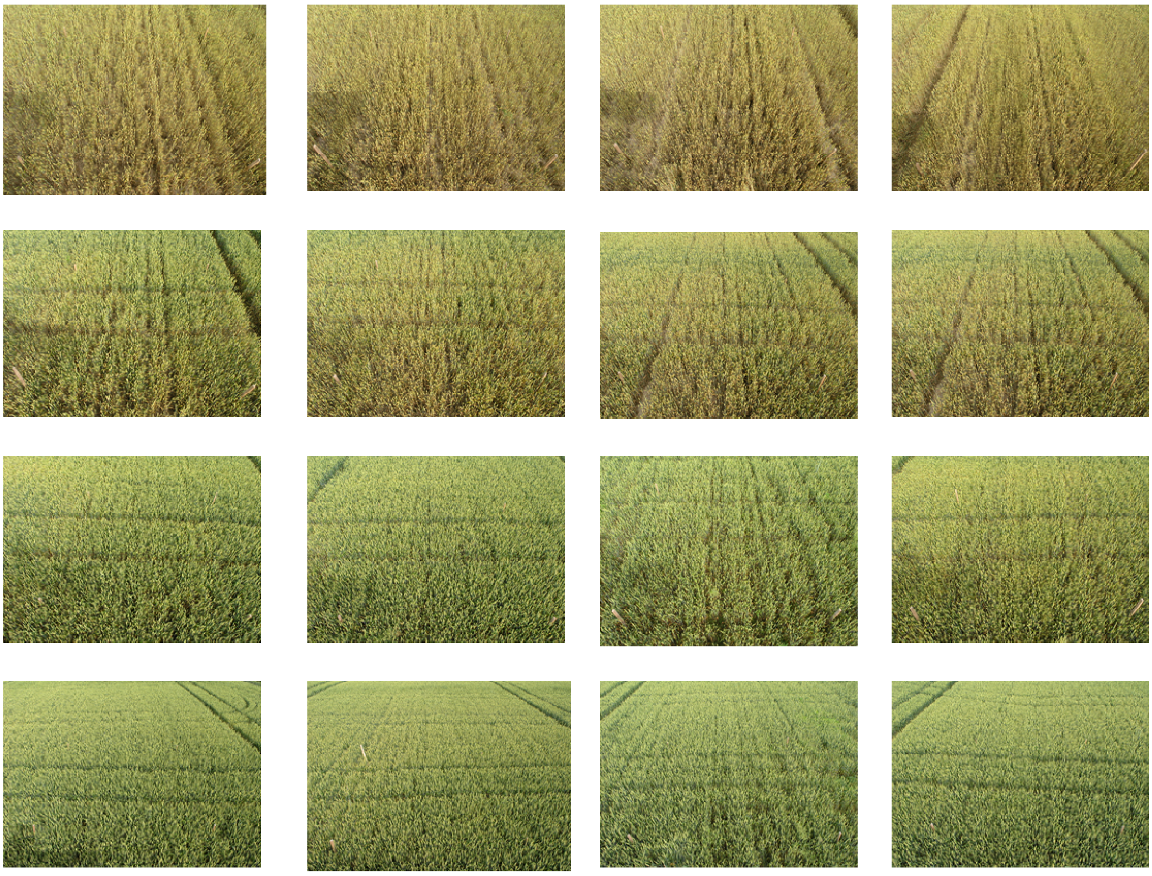


Figure 3.1 – Experiment plots (4 repeat plots per treatment) captured from the high during ripening stage, 2008 (from above N_0, N_{100}, N_{200} and N_{300} kg ha^{-1}).

4 MATERIALS AND METHODS

4.1 Location and research conditions

Research was conducted on experimental field within drained cropland used by Moslavka d.d. company in Western Pannonian subregion of Croatia near Park of nature Lonjsko polje (45°33´N, 16°31´E) (Figure 4.1.1). Terrain is flat with average elevation of 97.2 m. Region characteristic are small-holdings which dominate within rural land from one side, and hydro-ameliorated area with organized agricultural production within larger agricultural holdings (Figure 4.1.2). Experimental field is a part of a long-term research of influence of mineral nitrogen (N) fertilization on nitrogen use efficiency (NUE), crop yield and nitrate leaching within a framework of scientific project “Nitrogen fertilization acceptable for environment” (Project coordinator: Milan Mesić; 178-1780692-0695, funded by Ministry of Science, education and sports, MZOS).

Figure 4.1.1 - Area of Park of Nature Lonjsko polje with location of experimental field,

(Source: www.pp-lonjsko-polje.hr).

Figure 4.1.2 – Satellite images of field experiment: (a) cropland near Park of nature Lonjsko polje; (b) experiment during 2010; (c) experiment during 2008, *(Source: Google Earth, 2010).*

The area has a temperate continental climate, with 10.7 °C of annual mean temperature (Figure 4.1.4) and the annual amount of rainfall of 865 mm for the reference period 1965-1990 (Figure 4.1.3). Average annual cycle of mean monthly temperature for years 2008 and 2010 had the same shape as for the reference period 1965-1990 (Figure 4.1.4). When analyzing vegetation period, the largest differences in warm 2008 compared to 1965-1990 were in winter and late spring which were warmer from 2.4°C to 3.4°C per month. Higher monthly temperatures appeared during whole vegetation growth. Colder winter than average was recorded in 2010. However, the rest of the vegetation period was warmer than average (0.6 – 3.4°C). Mean annual temperature was 1.9°C and 0.8°C higher in 2008 and 2010, respectively, compared to the reference period. Significant differences in monthly precipitation between 2008, 2010 and the reference period were recorded during most of the vegetation period (Figure 4.1.3). Both 2008 and 2010 were characterized by high annual variability and intra-annual redistribution of monthly amounts. It is interesting that both years had almost similar shape of the average annual precipitation cycle, but 2008 had much lower amounts during spring and summer months. The shape of the average annual precipitation cycle of 1965-1990 period indicated optimal conditions for winter crops growth. Annual precipitation amounted 659 mm and 1120 mm for 2008 and 2010, respectively, indicating significant difference between those two years and compared to the reference period (865 mm). The minimum amount of precipitation in 2008 and reference period was recorded in February, while 2010 had minimum in early autumn. Maximum annual precipitation was recorded in June for all three periods but with high inter-annual differences. Increase in monthly precipitation during 2010 compared to other two periods was found in all months except March and April when analyzing winter wheat vegetation period. The most pronounced decrease in 2008 was recorded in winter and spring months. According to presented meteorological data it can be concluded that year 2008 was much drier than reference period and 2010, while 2010 can be pronounced as very wet year compared to the reference and 2008. Differences in soil water balance (after Thornthwaite) during years of investigation and the reference period generated from meteorological data measured at the nearest meteorological station are shown in Figure 4.1.5. The annual amount of evapotranspiration was 653 mm, 565 mm and 721 mm for years 1965-1990, 2008 and 2010, respectively. Soil water balance for two extreme years (2008 and 2010) showed large difference in seasonal pattern of water surplus and water deficit. Water deficit in 2008 was the main problem which caused drought during critical crop phenophases. Water deficit caused by precipitation lower than normal combined with high mean monthly temperatures was recorded in May, June, July and August. The main characteristic of the wet year 2010 was increased water surplus in months usually having optimal water supply, which was the reason for water stagnation in soil profile. There was no water deficit in soil during annual cycle. According to the average values for the reference period, water shortage was recorded only in August. Water surplus averaging 38.3 mm from January to April, and it was much lower than one recorded in 2010 (56.6 mm).

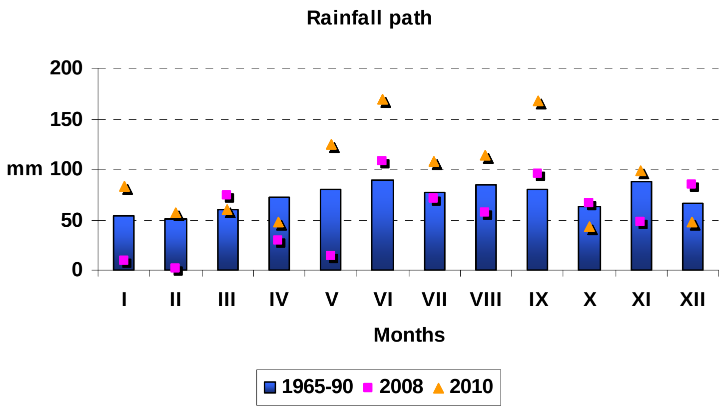


Figure 4.1.3 – Monthly precipitation during year 2008, 2010 and averaged across long-year period from 1965-1990 (meteorological station Sisak).

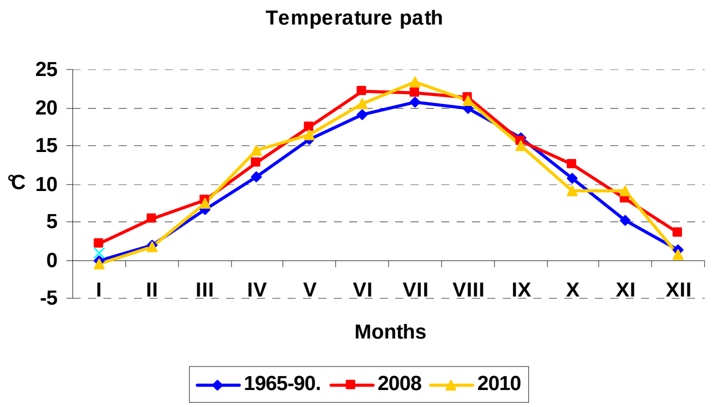


Figure 4.1.4 – Mean monthly temperature during year 2008, 2010 and averaged across long-year period from 1965-1990 (meteorological station Sisak).

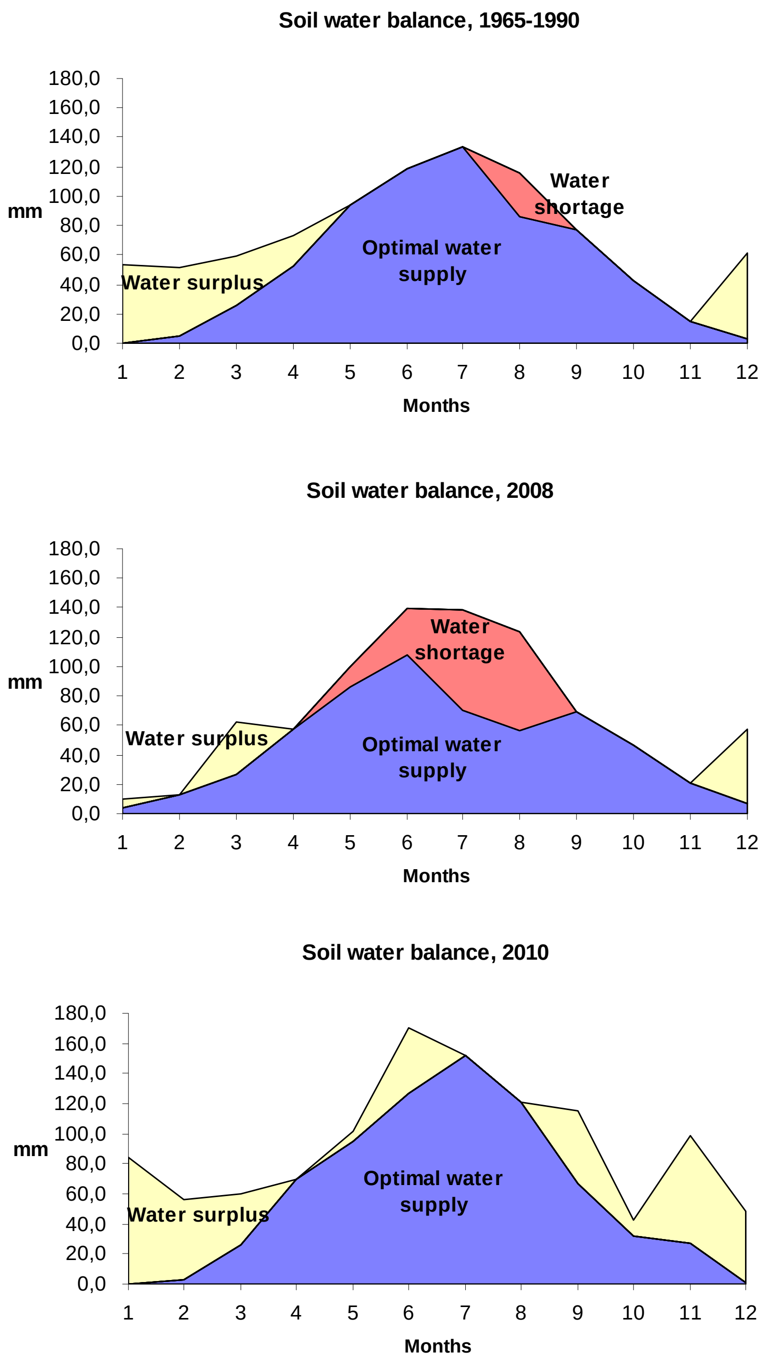


Figure 4.1.5 – Soil water balance (after Thornthwaite) for meteorological station Sisak for reference period 1965-1990, and years 2008 and 2010.

The soil type of trial site is distric Stagnosol (Figure 4.1.6). Sufficient moisture is present in upper part of soil profile as result of precipitation and stagnation of water. Groundwater occurs under 175 cm below soil surface. Precipitation water periodically stagnates on illuvial horizon which was the reason for installing pipeline drainage system across the experiment area.

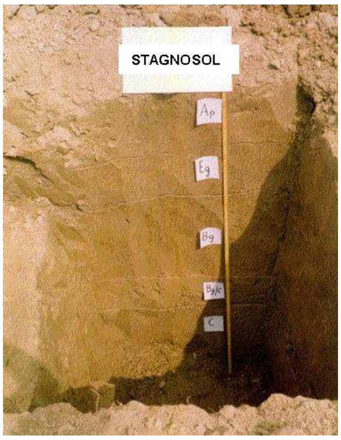


Figure 4.1.6 – Soil profile of Stagnosol – experimental field, 1996.

Besides soil physical properties that favorize water stagnation in upper layers and low soil organic matter content, the main factor that limits crop yield is low soil pH value. Soil is acid in first two horizons, but pH increases with depth according to carbonates accumulation. Figure 4.1.7 shows within-field spatial variability of soil pH values (0-30 cm depth) measured in 2010 after winter wheat harvest. Soil pH map was generated by ordinary kriging with sampling density of 15 x 15 m regular grid (ArcView, ESRI, 2006). Results show that field experiment is not uniform with respect to acidity (interpolation range of soil pH: 3.31 – 6.91) and that treatments differ between themselves in soil pH value range. In addition, each treatment consists of areas with differing lime needs. Sequence of changes in pH values was influenced by increasing N rates, influence of lime materials on treatment with 250 kg N per hectare (VIII.), effect of parent substrate on treatment with 100 kg N per hectare as result of drainage, and canal deposit on northeastern side of field. Low soil pH values on N treatments are mostly result of long-term mineral nitrogen fertilization. According to the project objective, calcification of all experiment area was excluded as measure for improving crop yield, except for one treatment which combined N fertilization with dolomite.

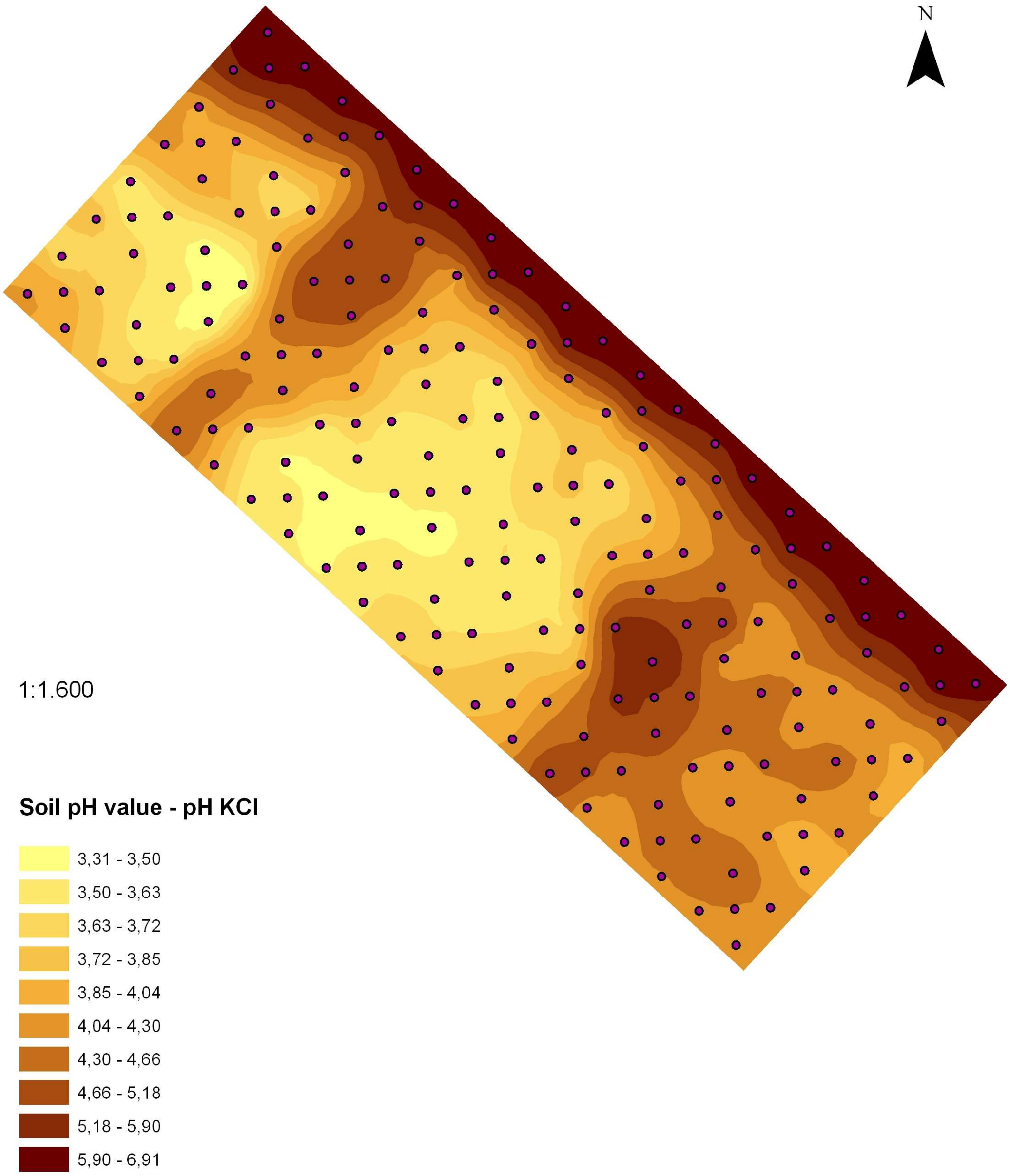


Figure 4.1.7 – Soil pH value measured on field experiment after winter wheat harvest, 2010.

4.2 Experimental design

The trial, with total area of 4 ha, was established as block design with different mineral nitrogen fertilization treatments and 4 replications. Parcel dimension is conditioned by distance between drain pipes. Each treatment area includes two drain pipes. Dimension of each trial treatment is 30 x 130 m including blank space, and 26 x 26 m for replication parcel. Fertilization and seeding practice is implemented on total area of each variant.

Fertilization treatments included (Figure 4.2.1, 4.2.2):

- **I. Control – no fertilization,**
- **II. N_0PK,**
- **III. N_{100}PK,**
- **IV. N_{150}PK,**
- **V. N_{200}PK,**
- **VI. N_{250}PK,**
- **VII. N_{250}PK + Phospho-gypsum,**
- **VIII. N_{250}PK + Dolomite,**
- **IX. N_{300}PK (kg N ha^{-1})**

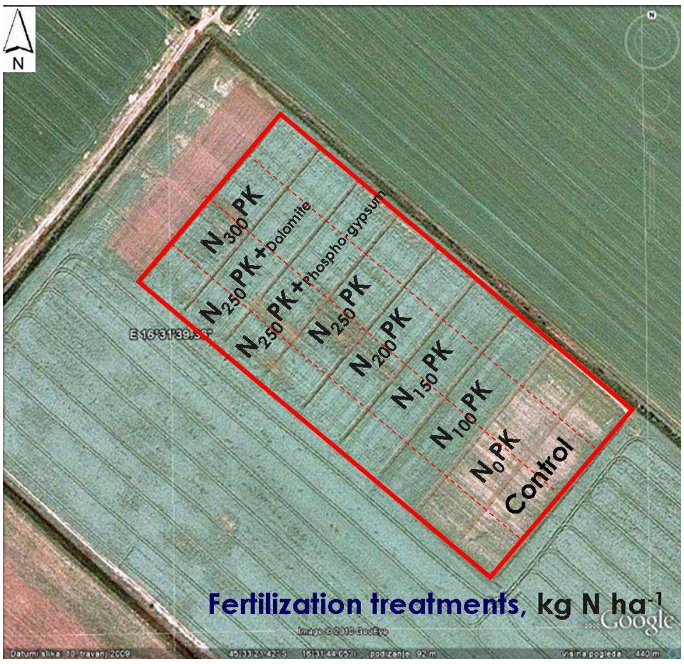


Figure 4.2.1 – Field experiment with different nitrogen fertilization treatments *(Source: Google Earth, 2010).*

Field experiment presented in dissertation included growing years 2008 and 2010 with winter wheat (*Triticum aestivum* L.) as a test crop with standard agro-technical management. Basic fertilization as 2/3 of total amount of PK mineral fertilizer was applied during ploughing up to depth of 25-30 cm, while other 1/3 of total amount with 30 % of N was applied directly before sowing. Nitrogen topdressing was performed in three amounts using calcium-ammonium-nitrate (CAN): I. 25 %, II. 25 % and III. 20 %. The first topdressing was applied in beginning of spring tillering, second and third during stem extension. Fertilization for winter wheat amounted for 500 kg of complex mineral fertilizer NPK 10-30-20 for treatment with 200 kg N ha^{-1}, as for all treatments with higher amounts of mineral N (N_{250} and N_{300}). Within 500 kg NPK 10-30-20 applied, soil was treated with 50 kg N, 150 kg P and 100 kg K, which was the reason for applying 334 kg of triple super-phosphate (150 kg P_2O_5) and 170 kg of 60 % potassium chloride (102 kg K_2O) for treatments II. N_0PK, III. N_{100}PK and IV. N_{150}PK. Correction of N fertilization amounts to assess exact values for N was made using single fertilizers (urea, CAN). Calcification (3 t ha^{-1} every third year) was included only in one fertilizer treatment (VIII. N_{250}PK + dolomite). Sowing of winter wheat was carried out with *Fiesta* cultivar (300 kg seed ha^{-1}) and *Lucija* cultivar (280 kg seed ha^{-1}). Weed control was performed using herbicide *Tena* in amount of 1.5 kg ha^{-1}.

Figure 4.2.2 – View from the control treatment on the field experiment during winter wheat tillering stage, 2008.

4.3 Plant material

The research was carried out at the two winter wheat growth stages during growing years 2008 (*Triticum aestivum* L. – cultivar "Fiesta") and 2010 (*Triticum aestivum* L. – cultivar "Lucija") to explore the potential for detection of the plant nitrogen status in focus, thus creating basis for a yield compensating nitrogen application strategy. The reason for choosing winter wheat as a test crop is fact that this plant is a good indicator of nitrogen stress symptoms expressed as loss of green color in the leaves, decrease of leaf area and intensity of photosynthesis (Kastori et al., 2005). Agrotechnical practices managed by Moslavka holding included choice of cultivar, which was the main reason for having different winter wheat cultivars in crop rotation defined by project methodology. "Fiesta" is medium early, and "Lucija" early maturing cultivar, both with similar morphological and yield performances. Beside the observed climatic differences between two growth years, cultivar properties were included as well as external factor affecting investigated agronomic and crop spectral variables.

The nitrogen plays main role in wheat nutrition because of its importance in protein and nucleic acid synthesis. Wheat properties are mainly caused by effect of genetic factors in interaction with environment (Balogh et al., 2006). So, in order to evaluate the degree of nitrogen stress measured through spectral reflectance, destructive chemical analyses were carried out and used as a measure of the real nitrogen status to which predictions were compared. The total N content represents indicator of N accumulation in plant (Desai and Bathia, 1978) indicating root system activity and translocation of organic and inorganic matter to top of plant. Loss of chlorophyll which content is approximately proportional to leaf nitrogen content is associated to environmental stress (Hendry and Price, 1993). Environmental stress, among many other factors in the outdoor conditions, is accomplished through experimental design and different nitrogen fertilization rates.

Winter wheat leaf sampling with conducting non-destructive field measurements (hyperspectral data and CCI readings) was carried out during stem extension (F8-9: flag leaf visible) and heading stage (F10.5: heading complete) (Feekes` scale) (Figure 4.3.1, 4.3.2), and directly before harvest for grain analysis of each vegetation year. Samborski et al. (2009) reported in their review article that Chl measurements for small grains are most optimal in the phenological period from tillering/stem extension to heading stage for in-season N application.

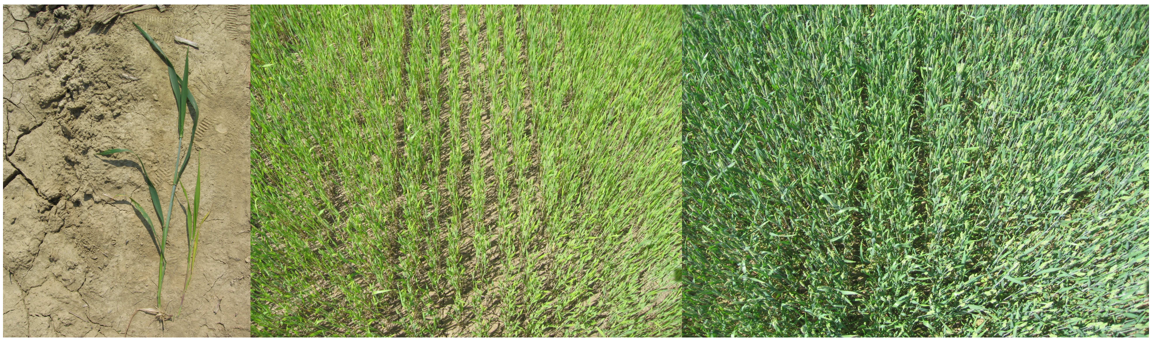


Figure 4.3.1 – Winter wheat during stem extension growth stage (2008 – "Fiesta"). Comparison of Control treatment (left) and N_{250}PK + Dolomite treatment (right).

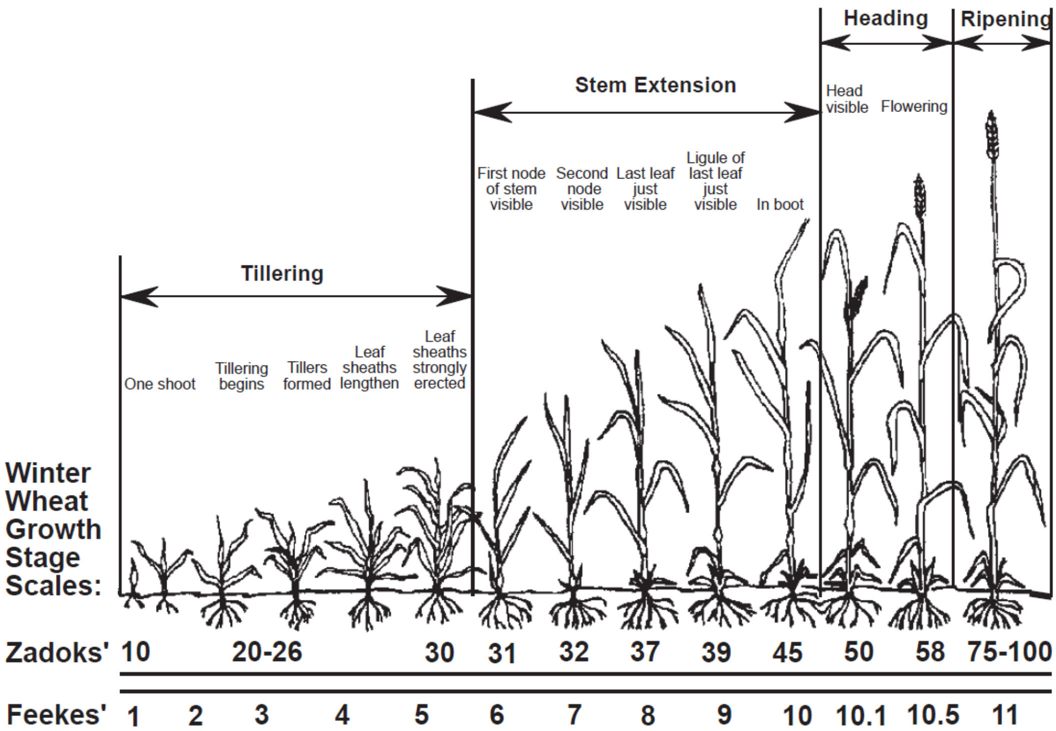


Figure 4.3.2 - Growth stages of winter wheat according to the Zadoks and Feekes scales

(Source: http://www.ext.vt.edu/pubs/grains/424-026/424-026.html#L4).

Yield and grain protein concentration depend primarily on nitrogen supply before flowering: potential number of grains is determined at this stage and the remobilization of nitrogen stored in vegetative organs before flowering and transferred to grains after this stage accounts for 70 % of the N uptake in the grains at harvest on average (MacKown and van Sanford, 1988). Thus, for winter wheat, the measurements of CCI aiming at quantifying the crop nitrogen fertilizer requirements are generally realized before flowering, at Zadoks growth stage 30–49 (Fox et al., 1994). Nevertheless, several studies have demonstrated the interest of chlorophyll meter measurements at later stages for the prediction of grain yield and grain protein concentration [GS65: Reeves et al. (1993), GS71 beginning of the milk stage: Le Bail et al. (2005), grain filling period: Benedetti and Rossini (1993)]. Osbourne et al. (2002) reported that estimation of corn grain yield was best accomplished by using spectral data from the late July sampling date.

The N concentration of plants is highest at early growth stages and decreases continually up to the stage of senescence, with this reduction in N content typically being interpreted as a dilution effect of growth through differential tissue N contents (Mistele and Schmidhalter, 2008). Stem extension is the most rapid period of vegetative growth where plant builds a structure for producing carbohydrate to fill the grain – flag leaf makes up approximately 75 % of the effective leaf area that contributes to grain fill (Beuerlein, 2001). This stage is also very sensitive to nitrogen deficiencies, thus providing good basis for discriminating crops with different nitrogen status using in-field hyperspectral sensing. Figure 4.3.3 illustrates the large N uptake from the beginning of April through the first two weeks of May for a well-fertilized crop grown under Virginia climatic conditions (Alley et al., 1996). During this phase nitrogen fertilizer management must provide enough nitrogen for the crop development in order to have adequate leaf area for producing profitable yields. Also, there is very little chance for leaching loss of N fertilizer applied near the beginning of this growth phase due to the extensive nature of the wheat root system by first part of stem extension, relatively high rates of evapotranspiration, and the large amount of N uptake during this time period. On the other hand, nitrogen uptake during the grain-fill period (Figure 4.3.3, late May through June) is relatively low compared to uptake during the stem elongation phase of growth. Plant tissue N is mobilized and translocated to the grain during this period with only small additions coming from available soil N. Foliar N applications at this growth stage have been shown to enhance grain protein levels.

Therefore, two distinct winter wheat growth stages according to different N distribution trends in plant tissue were chosen for detecting N stress and predicting yield components using leaf hyperspectral reflectance analysis.

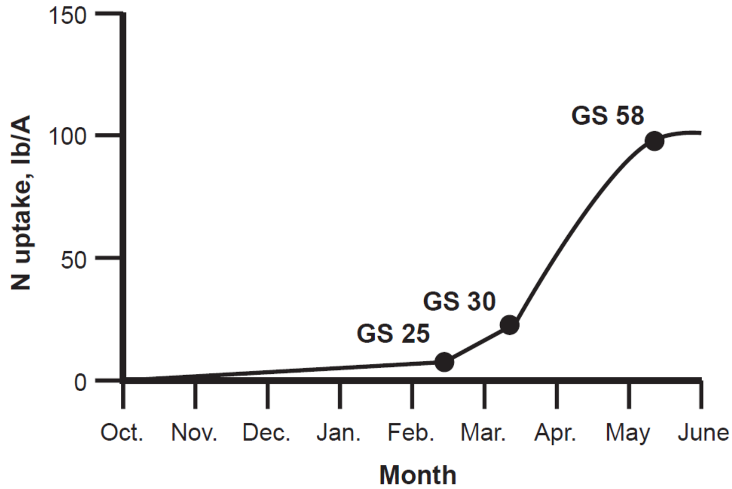


Figure 4.3.3 – Nitrogen uptake pattern for winter wheat grown in the Coastal Plain region of Virginia *(Source: http://www.ext.vt.edu/pubs/grains/424-026/424-026.html#L4).*

4.4 Time schedule

Following activities were performed within field work during growing years 2008 (Figure 4.4.1) and 2010 (Figure 4.4.2):

11 November 2007 – basic fertilization for winter wheat

13 November 2007 – winter wheat sowing - cultivar "Fiesta"

03 March 2008 – first N topdressing

09 April 2008 – second + third N topdressing

09 May 2008 – non-destructive field measurements (CCI, leaf spectra) + plant sampling

10 June 2008 – non-destructive field measurements (CCI, leaf spectra) + plant sampling

11 July 2008 – measurements of crop density + plant sampling

13 July 2008 – winter wheat harvest + plant sampling

Figure 4.4.1 - Field work during growing year 2008.

17 October 2009 – basic fertilization for winter wheat

18 October 2009 – winter wheat sowing - cultivar “Lucija”

20 March 2010 – first N topdressing

24 April 2010 – second + third N topdressing

07 May 2010 – non-destructive field measurements (CCI, leaf spectra) + plant sampling

14 May 2010 – non-destructive field measurements (canopy spectra)

05 June 2010 – non-destructive field measurements (CCI, leaf spectra) + plant sampling

12 July 2010 – measurements of crop density + winter wheat harvest + plant sampling

Figure 4.4.2 - Field work during growing year 2010.

4.5 Plant sampling, measurements and methods

Plant sampling scheme is described as discrete point sampling which uses an understanding of field variability to reduce the number of samples while still recognizing zones of differing N status due to fertilization treatments. In-season plant samples composed of 10 flag leaves were taken according to grid sampling design from each of 36 experimental plots from crop area of 1 m^2 in order to determine leaf spectral reflectance, total nitrogen (TN %) content in dry leaf and fresh leaf chlorophyll (Chl) content. Precise position of samples was recorded by GPS (Garmin; ± 4 m). Before harvest, whole winter wheat plants were sampled from the grid based area of 1 m^2 on each plot to determine crop density and yield variables. After harvest, grain yield was measured and standardized to dt ha^{-1} of total dry matter.

4.5.1 Field measurements

4.5.1.1 Leaf spectral reflectance measurements

Ground-based measurement of spectral reflectance was acquired at two winter wheat growth stages – stem extension (F8-9) and heading (F10.5) during years 2008 (Cultivar "Fiesta") and 2010 (Cultivar "Lucija") (Figure 4.5.1.1.2) from 10 flag leaf samples per area of 1 m^2 representing a sub-sample of the experiment repetition parcel (total of 360 leaf spectra per stage). The same samples were used for standard TN content analysis in dry leaf tissue and *in-situ* CCI readings in fresh leaf tissue, which represented reference data used for calibrating hyperspectral sensor. Non-destructive proximal *in-situ* stationary quantitative measurements of leaf reflectance were performed using portable field spectroradiometer FieldSpec®3 (ASD Inc., USA) with wavelength range from 350 to 1050 nm, sampling interval of 1.4 nm and spectral resolution of 3 nm at 700 nm. Leaf samples were scanned with close proximate contact using a battery-powered hand-held fiber-optic probe (connected to the spectroradiometer with a fiber optic cable) with built-in artificial light source which allows ideal control of viewing and illumination geometry, important for biochemical modeling in order to evaluate predictive ability of VNIR spectroscopy in assessing nitrogen status in wheat (Figure 4.5.1.1.1). Quartz halogen lamps of 200-500 W power with a spectral temperature of 3,400°K were used to provide a light spectrally similar to sunlight, which is usually recommended for making spectral measurements (Curtiss and Goetz, 1994).

A spectroradiometer measures, as a function of wavelength, the energy coming from an object within its view (Lillesand et al., 2004). It is an optical spectrometer that produces electrical signals, which correspond to radiant flux energy falling on its detectors for a series of discrete wavelength intervals. Spectroradiometer uses single photoelectric detectors over which a spectrum is scanned and/or an array of detectors aligned in the spectrum path. Photoelectric detector current responds linearly to radiant flux energy while voltage responds logarithmically. Instrument has built-in electronics for converting the analogue signal to digital. The signal output of a spectroradiometer may be calibrated to NIST traceable standards to produce measurements with Radiance or Irradiance units.

Through a fiber-optic input, this system acquires a continuous spectrum by recording data in 700 narrow bands simultaneously (over the range 350 – 1050 nm). The unit incorporates a built-in notebook computer, which provides for flexibility in data acquisition, display and storage. As a standard feature, reflectance spectra are displayed in real time. Calculation of band ratios and other computed values was also performed in this research and will be explained in following chapters.

Using a spectroradiometer to obtain spectral reflectance measurements is a three-step process. First, the instrument is aimed at a calibration panel (NIST-traceable) of known, stable reflectance (Spectralon®, Labsphere, Sutton, NH), which is a hard and durable white unglazed ceramic surface having a reflectance averaging about 98.2 %, varying with wavelength from 95.0 to 99.3 %. The purpose of this step is to quantify the incoming radiation, or irradiance, incident upon the plant leaf or canopy. The instrument is then suspended over the plant leaves and the reflected radiation is measured. Panel measurements were taken before initial leaf readings and repeated approximately every 15 minutes. Vegetation radiance measurement was taken by averaging 10 scans at an optimized integration time, with a dark current correction at every spectral measurement. Spectrum averaging was used to reduce the noise in the desired spectral signal. Finally, the spectral reflectance of the object is computed by rationing the reflected energy measurement in each band of observation to the incoming radiation measured in each band. The term reflectance factor is used to refer to the result of this computation. A reflectance factor is defined formally as the ratio of the radiant flux actually reflected by leaf surface to that which would be reflected into the same sensor geometry by an ideal, perfectly diffuse surface irradiated in exactly the same way as the sample (Lillesand et al., 2004).

Figure 4.5.1.1.1 – Proximal reflectance measurements of winter wheat flag leaves acquired by field spectroradiometer (F8-2008).

Figure 4.5.1.1.2 – Winter wheat during stem extension (F8) and heading (F10.5) growth stage, 2008.

4.5.1.2 Chlorophyll concentration index (CCI) readings

Leaf chlorophyll concentration index was determined using portable CCM-200 Chlorophyll Content Meter (ADCBioscientific Ltd., England) on the same 10 fully expanded flag leaf samples per 1 m^2 scanned with the spectroradiometer, which means total of 360 CCI readings per growth stage during years 2008 and 2010. The midpoint of the last fully developed leaf was found to be the best position on the winter wheat plant on which to take chlorophyll meter readings (Hoel, 1998). After selecting the flag leaf, readings were taken from a sample area of a 1 cm diameter circle half the distance between the leaf tip and the collar and halfway from the leaf margin to the mid-rib (Figure 4.5.1.2.1). The mean of 3 readings was obtained for each flag leaf.

In-situ CCI measurements in fresh leaf tissue represented reference data used for calibrating hyperspectral sensor, and biophysical feature in modeling relationship with leaf spectral data. Since chlorophyllmeter uses leaf spectral response, information obtained by this instrument is similar to spectral data from spectroradiometer, considering specific wavebands in red and infrared regions.

Figure 4.5.1.2.1 – Chlorophyll measurements, 2010.

Since most leaf N is contained in chlorophyll molecules, there is a close relationship between leaf N and leaf chlorophyll content. This strong positive relationship is the basis for predicting crop N status by measuring leaf relative chlorophyll content (Francis and Piekielek, 1998). Chlorophyll as quantified by the CCM-200 chlorophyll meter represents a unitless relative measurement of leaf chlorophyll content. It is theoretically possible to convert the meter readings to a measure of specific chlorophyll content. However, this is not necessary for N management considerations. Chlorophyll meters have their greatest sensitivity in the deficient to adequate range of N nutrition. As such, the meter cannot indicate how much excessive N is available to a crop. Its strength lies in measuring a relative difference in crop N status and the ability to detect the onset of an N stress before it is humanly visible.

The chlorophyllmeter makes instantaneous, rapid and non-destructive readings on a plant based on the quantification of light intensity (peak wavelength: 653 nm: red LED) absorbed by the tissue sample. A second peak (peak wavelength: 931 nm: infrared LED) is emitted simultaneously with red LED to compensate the leaf thickness (Figure 4.5.1.2.2). Compared with the traditional destructive methods, this equipment might provide a substantial saving in time and costs.

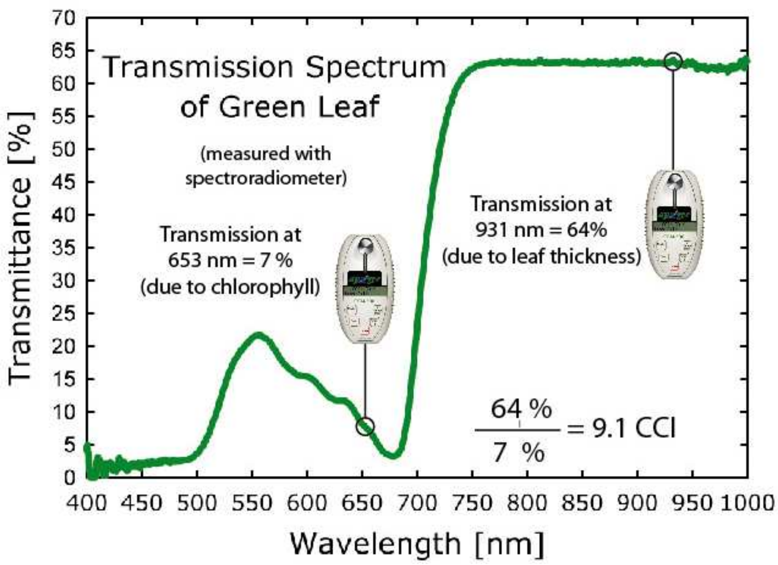


Figure 4.5.1.2.2 – Transmission spectrum of green leaf with absorption and reflection peaks of light *(Source: http://apogeeinstruments.com/chlorophyllmeter/ccm.html).*

4.5.1.3 Preharvest measurements and yield analysis

Directly before winter wheat harvest (Figure 4.5.1.3.1), crop density and yield components were measured from an area of 1 m^2 per repetition parcel. Total number of plants per 1 m^2, stem length, ears length, number and weight of grains per ear were determined. After harvest, grain yield was weighted and standardized to dt ha^{-1} of total dry matter, with determination of 1000-kernel weight and hectoliter mass.

Figure 4.5.1.3.1 – Winter wheat harvest – “Fiesta”, 2008.

4.5.2 Laboratory measurements

Standard destructive methods were used on average of 10 plant leaf samples and grain taken from 1 m^2/parcel. Laboratory analyses were used as reference measurements for calibrating spectroradiometer and making correlation analysis with spectral data acquired on the same leaf samples (Figure 4.5.2.1). Dry matter, water content and total nitrogen content (TN %) in dry leaf tissue and grain were determined.

Figure 4.5.2.1 – Analysis of total nitrogen (TN %) content in dry plant tissue using the dry combustion method with the Vario Macro CHNS Analyzer.

4.5.2.1 Samples preparation for chemical analysis

Winter wheat samples preparation for chemical analysis was performed according to HRN ISO 11464:2006. After the plant leaf sampling and determination of yield components, leaves and grain were dried in the oven at 60°C. After drying, samples were grinded with a knife mill (IKA, M20) and sieved through a<200 μm sieve (Retsch). Plant samples (powder) were than homogenized and each was subdivided by hand into two sub-samples and stored in plastic boxes (Figure 4.5.2.1.1). One sub-sample was prepared for laboratory analysis and the other for archive.

Figure 4.5.2.1.1 – Dried and grinded plant leaf and grain samples ready for the laboratory analysis.

4.5.2.2 Determination of total nitrogen by dry combustion method in leaf and grain

Total nitrogen (TN [% DM; g/kg]) content in plant leaf and grain was determined using the dry combustion method (elemental analysis) with the Vario Macro CHNS Analyzer, Elementar (2006) according to HRN ISO 13878:2004. TN content in plant samples was determined by heating the sample in oxidation column to a temperature of at least 900°C. Mineral and organic element compounds are oxidized and/or volatilized. Combustion products are mixture of different oxides: NO_x (NO and NO_2), N_2. In a reduction column (the temperature was 850°C at which Cu° was oxidized into Cu^{2+}) all nitrogen forms were transformed (reduced) into N_2. This gas was transported by the carrier gas helium (p (He) =

2.0 bar; *V (He)* = 500 *mL*/min) to the thermal conductivity detector (TCD) and after adsorption and desorption in specific columns, the TN content was measured. Pressure in the system was 1.24 bars. Standard sulphanilamide was used for daily calibration and daily factor calculation (99 %, Sigma; 16.27 % TN). The amount of test sample for analysis depended on the expected TN content. Approximately 100 mg ± 2 mg of oven-dried plant samples were weighted in thin foils (Figure 4.5.2.3.1). In solid sample, capsules were formed by pressing and compressing samples in folded thin boats. Samples were then placed in autosampler and were ready for analysis. Methods used in analysis were: method plant for plants (flow of O_2 up to 60 mL/min).

Figure 4.5.2.2.1 – Weighting of oven-dried plant samples.

4.5.3 Empirical extraction of information from real data

4.5.3.1 Derivation of vegetation indices from leaf reflectance

To estimate crop characteristics by remote sensing data, reflectance of selected wavelengths (mostly combination of red and infrared wavelengths) is transformed to vegetation indices (VIs) which are well used in praxis. These transformations are ratios or linear combinations of signals from radiometer bands. VIs provide highly correlated relationships with certain indices of crop development (wet and dry biomass, LAI, plant N status, Chl content, leaf water stress, yield), especially under stressed conditions of vegetation, and, hence, could provide important additional information for agriculture. Within the field, relative differences in the VI can show whether a crop is developing uniformly at any one time or over the growing season.

Using the high resolution reflectance spectra, among raw spectra and its 1st, 2nd derivatives and linearization as $\log(1/R_{reflectance})$, ratio- and difference-based vegetation indices were constructed using 231 spectral bands (over the range 400 – 1040 nm). Based on coefficients of correlation between each wavelength and crop parameter, and in consideration of spectral principle and commonly used VIs, the best feature bands (NIR = 785 nm, RED = 704 nm) and corresponding NDVI and RVI were determined (Table 4.5.3.1.1). Finally, the correlation of these particular VIs with winter wheat grain yield was evaluated to determine the strength of the agronomic parameter-spectra relationship. The Statistica software (Statistica 8.0, Statsoft Inc., USA) was used for statistical computations.

Table 4.5.3.1.1. Algorithms of hyperspectral vegetation indices analyzed for wheat.

Index abbreviation	Name	Algorithm	Reference
RVI	Ratio Vegetation Index (RVI)	R_{NIR}/R_{RED}	after Jordan, 1969
NDVI	Normalized Difference Vegetation Index (NDVI)	$(R_{NIR}-R_{RED})/(R_{NIR}+R_{RED})$	after Rouse et al., 1974

4.5.3.2 Calculation of relative indicators of agronomic performance

The N use efficiency of mineral N fertilization was calculated according to Raun et al. (2002) by equation:

$$NUE = (N\ removed_F - N\ removed_C)\ /\ Fertilizer\ N\ applied\ \%$$

F-fertilized crop; C-unfertilized control

N removed – grain yield x TN grain

According to Arnall et al. (2009), NUE, together with response index ($RI_{HARVEST}$) as agronomic indicator, has the ability to measure crop responsiveness to N fertilizer. NUE encompasses the effect of environmental factors such as temperature and moisture which highly influences N transformations and directly affects crop growth.

4.6 Statistical evaluation

Spectral data analyses by methods of multivariate statistics were performed to evaluate the prediction and discrimination results of nitrogen stress and status estimations from the spectral responses. Multivariate data analysis was chosen since it can be used in situations with numerous dependent and independent variables. Statistical evaluation was processed using spectroscopy software suite Unscrambler 9.7 (CAMO Software AS., Norway) and Statistica 8.0 (StatSoft, Inc., USA). Spectra were visually reviewed using software application ViewSpec Pro 4.07. (ASD, Inc., USA), and then formatted from .asd to .ASCII data format using ENVI (Research Systems, Inc., USA). Original spectral reflectance data were pretreated and transformed using various algorithms to avoid “shift” and “noise” phenomena and then included in statistic analysis. Preprocessing included first derivatives, second derivatives and linearization of raw spectra with Savitzky-Golay filter using a second-order polynomial for derivation and smoothing (le Maire et al., 2004). First-order derivatives involve the calculation of the slope of the spectrum (rate of change of reflectance with wavelength) (Liu et al., 2010). Second derivative was used as a mathematical treatment to correct the baseline effects and separate overlapping peaks (Moron et al., 2007). Derivatives are useful for reducing the effects of multiple scattering of radiation due to sample geometry, surface roughness and for locating the positions of absorption features on the spectra. Logarithmic transformation of reflectance [log (1/R)] or absorbance was used for visual spectra evaluation because it allows a simple correction of baseline drifts and approximates the actual behavior of various samples sufficiently well to be of practical use. Moreover, the absorbance peak shapes better maintain integrity important for evaluation of main differences in samples compared to the raw reflectance. Spectral bands up to 400 nm and beyond 1040 nm were removed due to large noise effect. Spectra were also resampled taking every third wavelength to reduce the amount of data and, thus, facilitate the complex statistical analysis.

Visible and near-infrared (VNIR) spectral reflectance of winter wheat leaf, the first derivative of reflectance, the second derivative of reflectance, the absorbance, band combinations (vegetation indices), and principal components of spectral data were chosen as independent data, and measured TN content in leaf and grain, CCI readings, yield and NUE as dependent variables. Independent data were calibrated to dependent data by means of linear modeling as Partial Least Squares Regression (PLSR) with leave-*n*-out cross validation and multiple linear regression (MLR) using Principle Component Analysis (PCA) for reducing data dimensionality and colinearity. The statistical models were validated using full cross validation, where each sample was used to test the estimation of the model by all the other samples. Discriminant analyses were performed to relate specific spectral bands to the fertilizer treatments. Spectral data patterns were processed using nonlinear modeling technique as Artificial Neural Networks (ANN) and than compared with linear models to define which model has the best predictive capability. Biophysical model for predicting the reference variables was also explained using specific vegetation indices. Models were validated for accuracy evaluation and predictive capability and then compared between themselves.

The main relations between spectral data and wheat variables at both investigated years were extracted and showed through performed statistical analysis. According to the better performance of cultivar “Lucija” (2010) model at growth stage F8 compared to the latter stage and cultivar “Fiesta” (2008) at both stages, the second year of research was selected for further analysis. The other reason for detailed analysis of only one year was to reduce the dimensionality of thesis volume. Visual spectra evaluation, CA and MLR were performed for all data, but only MLR results were shown in complete. Among four winter wheat variables, grain yield showed the best performance in both CA and MLR analysis, and it was selected for further regression (SLR – VIs, PLSR, ANN) and classification modeling (DA, CLA, ANN).

Statistical analyses of differences in leaf TN and relative Chl content, grain TN, NUE, yield and spectral parameters according to fertilization treatments for each growth stage and cultivar were computed by analysis of variance (ANOVA) (SAS 9.1, SAS Institute Inc., USA). It was important to define up to which N level spectral and agronomic features of winter wheat change which was a baseline for classification task. The significance test for overall statistics was performed at probability level of $p < 0.05$.

List of statistical analysis used in this research:

- Analysis of variance (ANOVA)
- Post-hoc test when F test is significant at probability level of $p \leq 0.05$ (Duncan`s multiple range test)
- Discriminant analysis (DA)
- Correlation analysis (CA)
- Cluster analysis (CLA)
- Modeling:
  - Principal component analysis (PCA)
  - Simple linear regression models (SLR)
  - Partial least square regression (PLSR)
  - Multiple linear regression (MLR)
  - Artificial neural networks – regression and classification (ANN)

4.6.1 Data manipulation and model development

Recent studies cited in this doctoral thesis have show that multivariate analytical techniques can prove quite useful in the interpretation of various forms of remotely-sensed data. Thus, multivariate linear and nonlinear modeling techniques have been used to explain the relationships between spectral data and winter wheat parameters, and, then compared for predictive and classification capability in this study (Figure 4.6.1.1). Spectral input variables consisted of 231 spectral data across full differently preprocessed spectral range taking every third wavelength, their principal components (PC) and vegetation indices. Spectral measurements were calibrated against samples with known chemical composition in order to extract the desired information: leaf TN, CCI readings, grain TN, yield and NUE as crop input variables. Calibration techniques were based on pattern recognition algorithms to capture the important variability in the data.

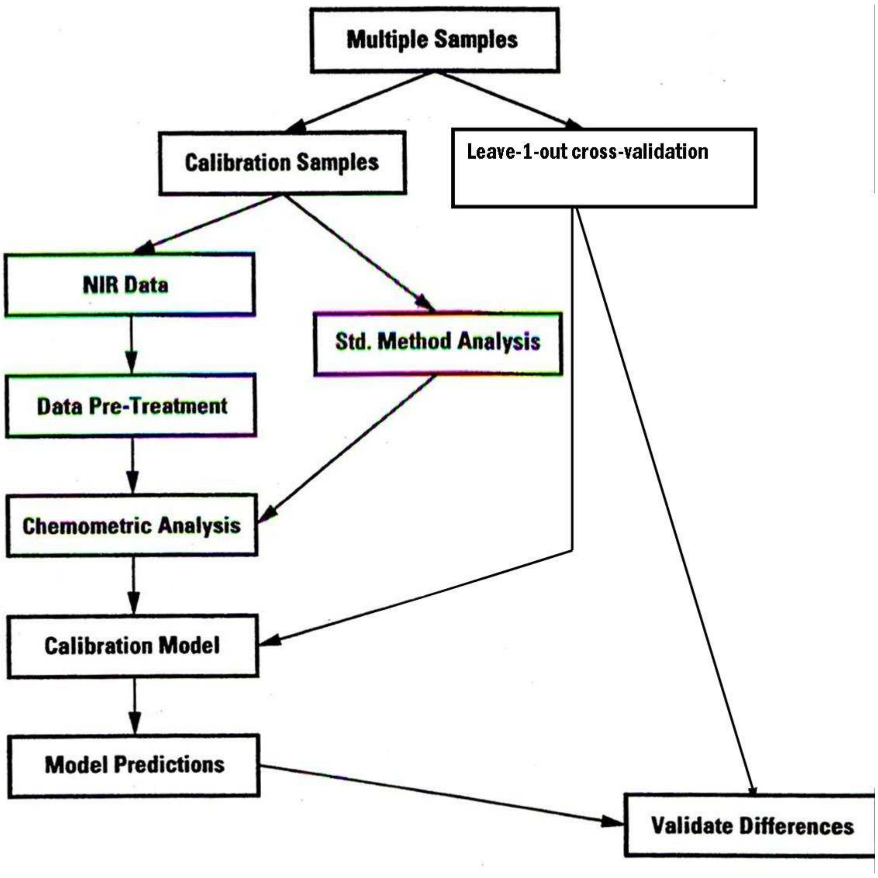


Figure 4.6.1.1 – Illustration of the biophysical and spectral modeling process (after Curtiss and Goetz, 2009).

Correlation analysis (CA) were done to determine if the reflectance at each wavelength was positively or negatively correlated with crop variables and to identify significant wavelengths as inputs for VIs computations, and thus, for simple regression models (SLR) where spectral indicators were regressed over wheat variables to predict N nutrition status and yield components in wheat. The best relationships derived from this correlation analysis were inverted to form an empirical model for winter wheat variables estimation from hyperspectral data.

Multiple linear regression (MLR) is one of the most commonly used linear methods to develop empirical models from large data sets and to predict crop biophysical variables in plants (Thenkabail et al., 2000). When the factors or independent variables are few in number and not significantly collinear (like PCs of spectra), and have a well-understood relationship to the responses or dependent variables, then MLR can be a good way to turn data into information, which was the case in this study. It is used for fitting models to continuous variables such as spectral data. Stepwise procedure was used to select the most effective spectral features capable of discriminating N treatment effects and predicting winter wheat variables (N, Chl, NUE, and yield) and to generate an equation for the optimum estimation of crop parameters from reflectance in selected wavelengths (Curran et al., 1992). Then, discrimination analysis (DA) was applied with a well-chosen subset of the selected PCs to classify samples by N application treatments and growth stage. The DA procedure generates one or more discriminant functions based on linear combinations of the predictor variables that provide the best discrimination between groups (Jensen et al., 2007). Factor scores acquired from the calculated PCs were used as predictor variables in a MLR modeling.

To avoid over-fitting (number of factors gets too large, poor prediction), some other “soft science” applications are used. The risk of overfitting arises when large numbers of independent variables are handled with a small number of samples. One of the methods is partial least squares regression (PLSR) with leave-1-out cross-validation or full cross-validation (each observation is used as a test set to validate the predictive model), analyzing numerous and collinear independent variables and response profiles. This method is well suited for calibration on a small number of samples with experimental noise in both biophysical and spectral data. In addition, the method can be used even if $n_{\lambda} > n_{obs}$ (Atzberger et al., 2010). Because each leaf sample is described by several hundreds of hyperspectral reflectance variables, PLSR, as a “full spectrum”, method seemed to be a reliable method to extract the relevant part of the information from very large data matrices which was proved by several studies (Hansen and Schjoerring, 2003; Nguyen and Lee, 2006). In this study, PLSR was used to evaluate and illustrate the correlation between the observed winter wheat variables and the spectral sensor results. PLSR provides a regression model where the entire spectral information is taken – in a weighted form – into account (Atzberger et al., 2010). It is bilinear technique that generalizes and combines features from principal component analysis and multiple regression. Its goal is to predict a set of dependent variables from a set of independent variables. This prediction is achieved by extracting from the predictors a set of orthogonal factors called latent variables or principal components that account for most of the variation in the response and have the best predictive power (Abdi, 2007). The number of PLSR factors used in each model was determined by full cross-validation. Principal components analysis (PCA) is used to avoid multicollinearity, and thus singularity in spectral data, which is the reason that regression approach is no longer feasible. PCA and PLS as full spectrum methods are widely used in chemometrics (Wold et al., 2001). PC are computed as certain linear combinations of the spectral amplitudes, and the responses are predicted linearly based on these extracted factors scores (Tobias, 1997). In this study, PCA was applied to all of the 231 spectral variables. This reduced the spectral information to few principal components (PLS – Unscrambler 9.7; NIPALS – Statistica 8.0). PCA found the set of directions in spectral space that explain the most variance. The first principal component is the combination that accounts for the largest amount of variance in the sample. The second principal component is uncorrelated with the first one and accounts for the next largest amount of variance. Successive components explain progressively smaller portions of the total sample variance, and all are uncorrelated with each other. Only those PC that individually captured more than 0.1% of the variance in the data set were retained (factors with eigenvalues greater than 1 – Kaiser criterion) (Statistica 8.0). All reflectance information was standardized prior to computing the principal components according to the procedure described by Thorp et al. (2008).

This effective method which reduces the number of input variables by removing the unnecessary or redundant information was also used in spectral analysis by artificial neural networks (ANN). These machine learning algorithms can be used in estimating various field and crop conditions from remotely-sensed images. This non-linear technique works with pattern recognition problems and learns by example. The neural network gathers representative data, and then invokes training algorithms to automatically learn the structure of the data (Statistica 8.0). The ability of ANN to associate complicated spectral information with target attributes without any constraints for sample distribution (Mather, 2000) make them ideal for describing the intricate and complex non-linear relationships which exist between canopy-level spectral signatures and various crop conditions (Kimes et al., 1998). In this study ANN was used to classify and predict continuous variables.

Regression type consisted of winter wheat variables as continuous targets and differently preprocessed and pretreated spectral data as continuous inputs. Classification type included fertilization levels as categorical target and spectral data as continuous inputs. So, the goal was to assign 36 wheat samples to 9 fertilization levels grouped in 3 categories based on spectral data and organized according to significantly different fertilizer treatments. The supervised back-propagation neural network model was trained using PC of spectral data as inputs and winter wheat variables (CCI readings, leaf and grain N content, NUE, yield) as an output. Spectral data were mean centered and normalized to a unit standard deviation. The spectra were randomly divided into training and testing sets with proportions of 50% and 50%, respectively. Models were trained in steps for different numbers of training cycles. The architecture of the ANN models used in this study was a fully connected feed-forward, with structure consisting of input neurons which number corresponded to the number of selected PCs representing the total variation of the target spectra, of one input layer, one output layer, and one hidden layer. Considering classification task of ANN, the output layer consisted of 3 neurons, one for each group of N treatment, and 36 neurons for identification of crop variables (Figure 4.6.1.2). Multi-layer perceptron (MLP) and radial basis function (RBF) network types with different activation functions and training algorithms were used, as described by Gautam et al., 2006.

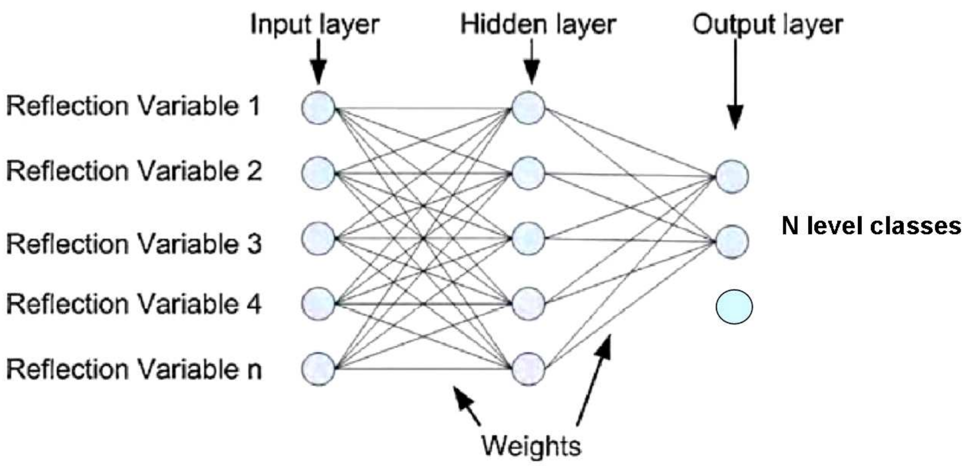


Figure 4.6.1.2 – Illustration of the neural network used in classification task. The NN shown has 1 hidden layer.

4.6.2 Performance analysis

Statistical parameters used for model accuracy and performance analysis were: correlation coefficient (r), coefficient of determination (R^2), root mean square error (RMSE) and sum of squares (SOS) error function and cross entropy (CE) error function for ANN learning algorithms. To assist with model comparisons, confidence limits (95%) for the regression parameters and the R^2 value were calculated for each model. The R^2 is a measure for precision and represents proportion of variability explained by the model. The RMSE is a measure of prediction accuracy and represents the residuals or the average difference between predicted and observed values. It is determined by means of cross-validation. The RMSE was calculated on the original unit basis. The SOS error function was used for assessing neural network performance and represents how close the ANN predictions are to the targets. The CE error function showed classification rate using ANN. In addition, the performance of each model was evaluated by plotting the predicted values against the observed values. For classification problems, the percent of predicted group membership was calculated for each class.

$$RMSE = \sqrt{\frac{\sum_{i-1}^{n}(ti - yi)2}{n}} \quad ,$$

Where *yi* is the predicted value of sample *i*; *ti* is the observed value of sample *i*; *n* is the total sample number. Models with the highest R^2 and the lowest RMSE were considered as the best to predict winter wheat variables.

$$Esos = \sum_{i-1}^{N}(yi - ti)2 \quad ,$$

where *yi* is the predicted value of sample *i*; *ti* is the observed value of sample *i*; *n* is the total sample number.

$$Ece = -\sum_{i-1}^{N} ti \ln\left(\frac{yi}{ti}\right)$$

5 RESULTS

5.1 Variation of agronomic variables

Mean values of TN content in winter wheat flag leaves sampled at stem extension - F8 and heading – F10.5 growth stage, during vegetation years 2008 (cultivar “Fiesta”) and 2010 (cultivar “Lucija”), are shown in Figure 5.1.1. Figure 5.1.2 overviews a comparison of mean values of TN content in winter wheat dry leaf tissue at both growth stages and grain tissue between vegetation years 2008 (cultivar “Fiesta”) and 2010 (cultivar “Lucija”).

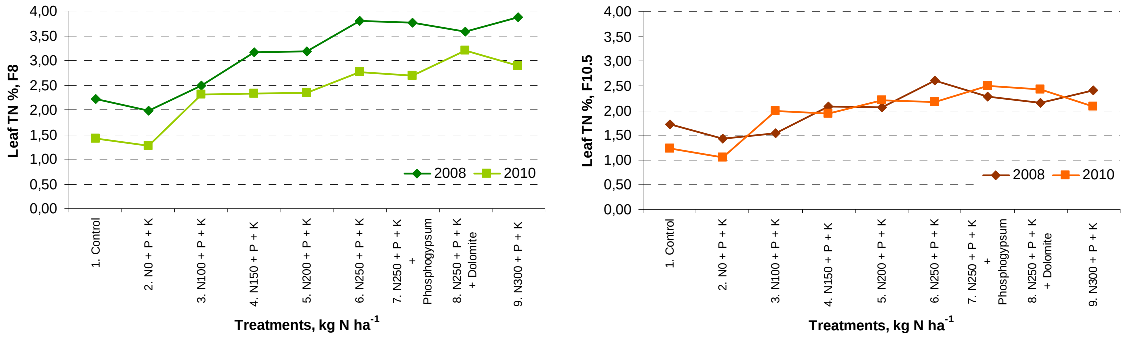


Figure 5.1.1 – Total nitrogen content (TN %) in winter wheat dry leaf tissue at stem extension - F8 (left) and heading – F10.5 (right) stage, in vegetation years 2008 and 2010.

When analyzing fertilization treatments pattern, flag leaf TN content varied across cultivars/years and growth stages. It was higher during stem extension (F8) stage 2008 compared to the same growth stage at 2010 which can be explained by extreme climatic conditions during crop development. Higher TN concentrations were recorded during 2008 with cultivar “Fiesta”. Differences in leaf TN content accounted for heading stage between cultivars and years were negligible because N movement from leaves to grain was not affected by unfavorable climatic conditions.

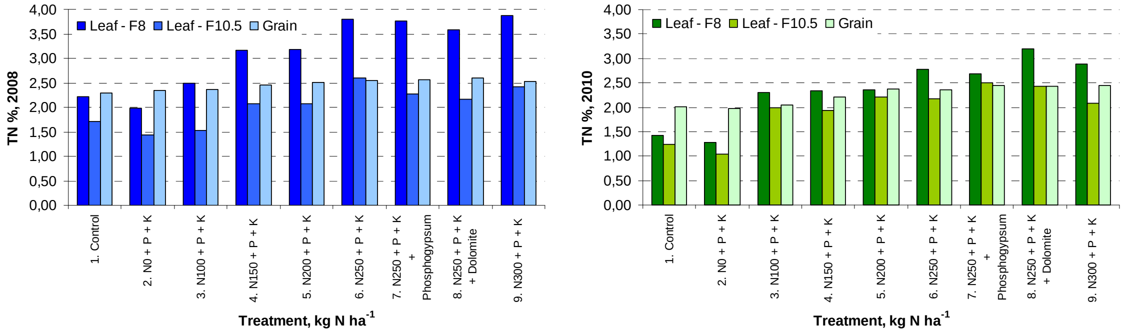


Figure 5.1.2 – Total nitrogen content (TN %) in winter wheat dry leaf tissue at two growth stages and grain tissue during vegetation years 2008 (left) and 2010 (right).

Chlorophyll readings, expressed as chlorophyll content index values, taken from winter wheat fresh flag leaf in stem extension - F8 and heading – F10.5 stage, during vegetation years 2008 and 2010 with cultivars “Fiesta” and “Lucija”, respectively, are shown in Figure 5.1.3. Data were averaged across sample set representing one treatment plot. Figure 5.1.4 shows a comparison of mean values of chlorophyll content index readings taken from winter wheat flag leaves at both growth stages during vegetation years 2008 and 2010, and cultivars “Fiesta” and “Lucija”, respectively.

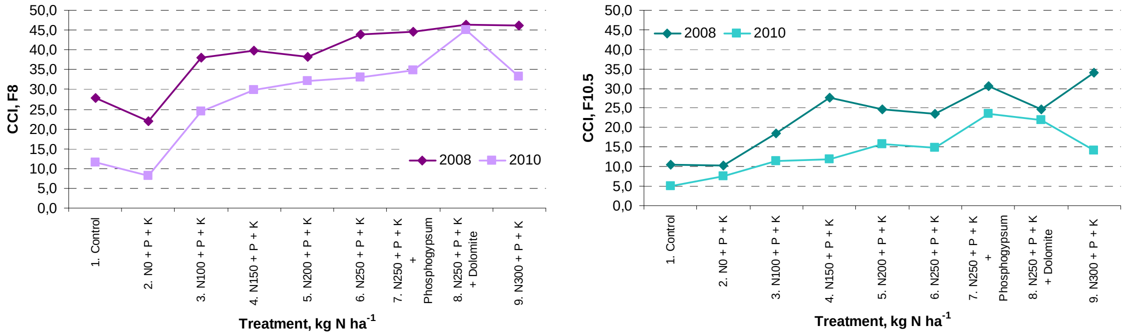
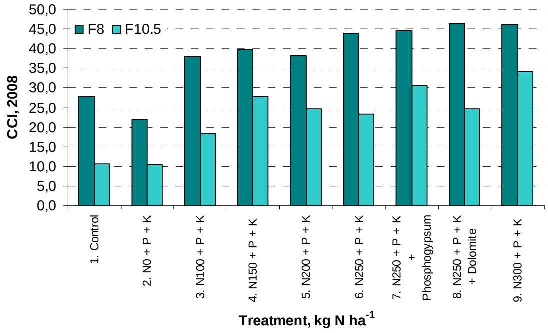


Figure 5.1.3 – Chlorophyll content index (CCI) readings of winter wheat fresh leaf at stem extension - F8 (left) and heading – F10.5 (right) stage, for vegetation years 2008 and 2010.

Cultivar “Fiesta” (2008) had higher mean values of CCI for both stages than cultivar “Lucija” which can be explained by unfavorable climate properties expressed by water surplus and lower crop development in 2010. However, pattern of differences between stem extension (F8) and heading (F10.5) was the same for both cultivars/years: CCI readings were higher for F8 then for F10.5 stage.



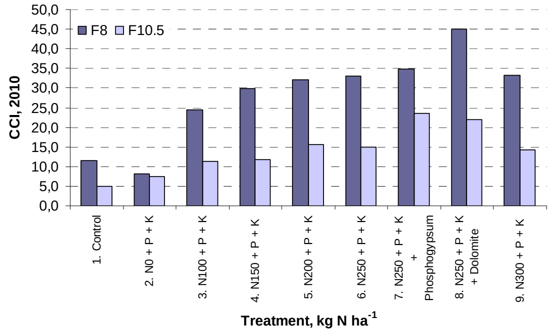


Figure 5.1.4 – Chlorophyll content index (CCI) readings of winter wheat fresh leaf at two growth stages during vegetation years 2008 (left) and 2010 (right).

Differences in mean grain TN content per each cultivar/year are shown in Figure 5.1.5. Grain TN content followed the same trend when comparing two vegetation years. Average concentration was lower in 2010 compared to 2008.

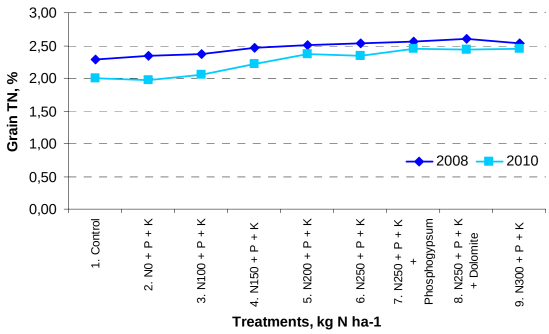


Figure 5.1.5 – Total nitrogen content (TN %) in winter wheat dry grain tissue in vegetation years 2008 and 2010.

Figure 5.1.6 represents dependency of nitrogen use efficiency (NUE) and grain yield of winter wheat on mineral nitrogen fertilization during two vegetation periods (2008 - cultivar “Fiesta” and 2010 - cultivar “Lucija”). The average yield for both cultivars and growing periods and across all N fertilization treatments was only 29.2 dt ha^{-1}. However, nitrogen was a key factor which defined level of winter wheat yield. Grain yield had in both years increasing trend with higher N fertilization levels, but it was lower in 2008 than in 2010 due to the canopy damages. The maximal average yield per treatment was 44.7 dt ha^{-1} (2010 – cultivar „Lucija“) for N_{250} level with phospho-gypsum. In 2010, NUE generally had decreasing trend in response to increasing amounts of fertilizer-N up to treatment VII after which it decreased again. Moreover, variable pattern of NUE was recorded in 2008 as well, except sharp increase was found in treatment VIII.

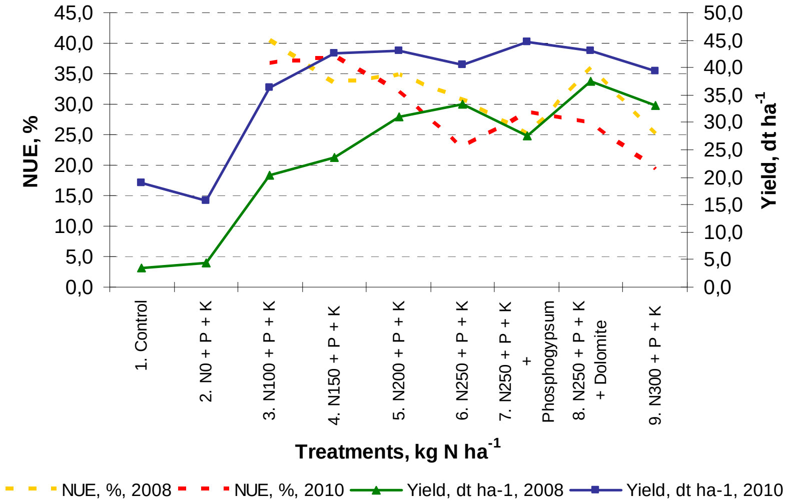


Figure 5.1.6 – Effect of mineral N treatments on winter wheat N use efficiency (NUE %) and yield (dt ha^{-1}) during two vegetation periods (2008 and 2010).

The corresponding descriptive statistics of the field and laboratory measured winter wheat variables for nine fertilization treatments, one measurement date per two growth stages and two winter wheat cultivars/years are summarized in Table 5.1.1. The table reports the minimum value (min), maximum (max), mean and standard deviation (SD) of the observations, as well as pooled data for overall measurements and analysis. The absolute range of analyzed variables varied widely: leaf TN content from 0.73 % to 4.27 %; CCI from 3.0 to 50.0; grain TN content from 1.89 % to 2.67 %; yield from 0.9 dt ha^{-1} to 50.1 dt ha^{-1}; and nitrogen use efficiency (NUE) from 3.1 % to 57.4 %.

Table 5.1.1. Descriptive statistics of the crop variables for nine fertilizer treatments, two growth stages and two winter wheat cultivars/years.

Crop variables	Pooled	Cultivar/Year		Growth stage		N fertilizer treatments								
		“Fiesta”/2008	“Lucija”/2010	F8	F10.5	I	II	III	IV	V	VI	VII	VIII	IX
Leaf TN (%)														
Mean	2.37	2.57	2.16	2.74	1.99	1.65	1.43	2.08	2.38	2.45	2.83	2.81	2.84	2.81
SD[1]	0.77	0.84	0.64	0.82	0.50	0.48	0.46	0.46	0.57	0.59	0.71	0.64	0.66	0.75
Max	4.27	4.27	3.54	4.27	2.94	2.63	2.30	2.90	3.62	3.87	4.27	4.20	4.05	4.26
Min	0.73	0.89	0.73	1.11	0.73	1.05	0.73	1.34	1.67	1.65	1.86	2.11	1.83	1.88
CCI														
Mean	25.8	30.6	21.0	33.3	18.4	13.7	12.0	23.1	27.3	27.6	28.8	33.4	34.5	31.9
SD	13.2	12.4	12.2	11.9	9.7	9.4	7.6	10.9	11.3	9.9	12.4	9.1	12.9	13.8
Max	50.0	50.0	49.5	50.0	46.6	35.6	28.9	42.5	46.1	39.7	49.2	46.9	49.9	50.0
Min	3.0	5.4	3.0	7.2	3.0	3.3	3.0	7.3	9.3	12.0	10.6	14.7	11.8	10.4
Grain TN (%)														
Mean	2.36	2.47	2.25	-	-	2.14	2.16	2.21	2.34	2.44	2.44	2.50	2.52	2.49
SD	0.20	0.13	0.21	-	-	0.17	0.21	0.21	0.16	0.10	0.16	0.09	0.11	0.05
Max	2.67	2.67	2.54	-	-	2.43	2.42	2.50	2.54	2.59	2.59	2.59	2.67	2.57
Min	1.89	2.22	1.89	-	-	1.93	1.89	1.93	2.08	2.34	2.08	2.30	2.34	2.41
Yield ($dtha^{-1}$)														
Mean	29.2	23.8	36.0	-	-	11.2	10.0	28.4	33.1	37.0	36.9	36.1	40.3	36.2
SD	13.3	12.6	11.2	-	-	8.9	6.6	9.9	11.1	7.5	5.8	10.0	7.3	5.2
Max	50.1	43.5	50.1	-	-	26.4	18.6	41.7	47.1	49.8	46.2	50.1	46.2	45.6
Min	0.9	0.9	12.3	-	-	2.5	0.9	17.4	15.6	26.7	27.1	25.3	23.2	31.2
NUE (%)														
Mean	30.8	32.2	29.4	-	-	-	-	38.6	35.6	33.5	26.9	26.9	31.6	22.4
SD	10.5	8.0	12.6	-	-	-	-	16.7	11.2	7.1	7.1	5.4	9.7	4.8
Max	57.4	49.8	57.4	-	-	-	-	57.4	50.8	42.9	37.3	37.9	43.0	26.1
Min	3.1	19.5	3.1	-	-	-	-	3.1	19.1	21.5	16.7	20.9	19.5	14.7

[1]SD, standard deviation

Table 5.1.2. Mean comparison for leaf nitrogen (N) content and chlorophyll content index (CCI), grain N content, yield and NUE between different N fertilizer treatments for two winter wheat cultivars under two growth stages, during 2008 and 2010, respectively.

Cultivar/Year	Treatment kg/ha		Growth stage				Yield variables		
			F8		F10.5				
	Code	N rate	Leaf TN %	CCI	Leaf TN %	CCI	Grain TN %	Yield $dtha^{-1}$	NUE %
„Fiesta“/2008	I	Control	2.22c[1]	27.8c	1.71bc	10.6c	2.29d	3.5d	-
	II	N_0PK	1.98c	22.0c	1.43c	10.3c	2.34cd	4.3d	-
	III	$N_{100}PK$	2.49c	38.0b	1.53c	18.4bc	2.37cd	20.4c	40.2a
	IV	$N_{150}PK$	3.16b	39.8ab	2.08ab	27.7ab	2.46bc	23.6c	33.3abc
	V	$N_{200}PK$	3.17b	38.2b	2.07ab	24.7ab	2.50ab	31.0ab	34.9abc
	VI	$N_{250}PK$	3.79ab	43.9ab	2.60a	23.4ab	2.54ab	33.3ab	30.6abc
	VII	$N_{250}PK+PG$	3.77ab	44.5ab	2.27a	30.5a	2.56ab	27.5bc	24.9c
	VIII	$N_{250}PK+D$	3.58ab	46.3a	2.15ab	24.7ab	2.61a	37.5a	36.1ab
	IX	$N_{300}PK$	3.87a	46.1a	2.41a	34.1a	2.53ab	33.1ab	25.3bc
„Lucija“/2010	I	Control	1.43c	11.6d	1.24d	5.0e	2.00c	18.9c	-
	II	N_0PK	1.27c	8.2d	1.05d	7.5de	1.97c	15.8c	-
	III	$N_{100}PK$	2.30b	24.5c	2.00c	11.3cde	2.05c	36.4b	37.1ab
	IV	$N_{150}PK$	2.34b	30.0bc	1.94c	11.9cde	2.21b	42.6ab	37.9a
	V	$N_{200}PK$	2.35b	32.0bc	2.22abc	15.7bc	2.37a	43.1ab	32.2ab
	VI	$N_{250}PK$	2.77ab	33.1bc	2.17abc	14.8bcd	2.35ab	40.5ab	23.1ab
	VII	$N_{250}PK+PG$	2.69ab	34.9b	2.51a	23.6a	2.45a	44.7a	28.9ab
	VIII	$N_{250}PK+D$	3.20a	45.0a	2.43ab	21.9ab	2.43a	43.1ab	27.0ab
	IX	$N_{300}PK$	2.88ab	33.4bc	2.09bc	14.2cd	2.45a	39.3ab	19.5b

[1]Means followed by the same letter within a column for each cultivar/year are not significantly different at the $p \leq 0.05$ level by Duncan’s Multiple Range test

Table 5.1.2. and Table 5.1.3. summarize the results of mean comparison for winter wheat crop variables: leaf nitrogen (N) content, chlorophyll content index (CCI), grain N content, yield, NUE and spectral parameters calculated as NDVI and RVI between different N fertilizer treatments for one measurement per two growth stages (F8 – stem extension; F10.5 – heading), during 2008 – cultivar "Fiesta" and 2010 – cultivar "Lucija". Analysis of variance of the N fertilization treatment on winter wheat variables showed that not all of nine treatments achieved significant differences (Table 5.1.2). The most obvious significant separation was detected between treatments without nitrogen (Control, N_0) and those with N excess (N_{250}, N_{300}). In most cases, treatments with 100, 150 and 200 kg N ha^{-1} were not significantly different between themselves, but they did differ from treatments with no N added or with those with 250 and 300 kg N ha^{-1}. NUE was only significantly different between the highest and the lowest values (N_{100} or N_{150} and N_{250} or N_{300}) for both investigated years and cultivars.

Table 5.1.3. Mean comparison for NDVI and RVI between different N fertilizer treatments for cultivar "Lucija" under growth stage F8, during 2010.

Cultivar/Year	Treatment kg/ha		Growth stage F8	
	Code	N rate	NDVI	RVI
„Lucija"/2010	I	Control	0.414c[1]	2.420d
	II	N_0PK	0.386c	2.267d
	III	N_{100}PK	0.598b	4.029c
	IV	N_{150}PK	0.643ab	4.616abc
	V	N_{200}PK	0.628ab	4.463bc
	VI	N_{250}PK	0.658a	4.878ab
	VII	N_{250}PK+PG	0.670a	5.085ab
	VIII	N_{250}PK+D	0.681a	5.264a
	IX	N_{300}PK	0.663a	4.959ab

[1]Means followed by the same letter within a column for cultivar/year are not significantly different at the $p \leq 0.05$ level by Duncan's Multiple Range test

Overall effect of different N fertilizer treatments, two winter wheat cultivars/years and two growth stages on leaf TN content, chlorophyll content index (CCI), grain TN content, yield and NUE is reported as mean comparison in Table 5.1.4. Mean differences between groups of N levels, cultivars and growth stages were statistically significant for every crop variable except NUE between cultivars/years. Although NUE did not differ significantly among two cultivars, mean values were slightly higher for 2008 compared to 2010. According to these results, means were later grouped into statistically different categories of N rates [I (Control, N_0); II (N_{100}, N_{150}, N_{200}); III (N_{250}, N_{300}) (kg ha^{-1})] which represented basis for classification analysis with spectral features.

Table 5.1.4. Mean comparison for leaf TN content, chlorophyll content index (CCI), grain TN content, yield and NUE for overall effects of different N fertilizer treatments, two winter wheat cultivars/years and two growth stages.

Treatment, kg/ha	Leaf TN %	CCI	Grain TN %	Yield $dtha^{-1}$	NUE %
I. Control	1.65d[1]	13.7e	2.14c	11.2d	-
II. N_0PK	1.43d	12.0e	2.16c	10.0d	-
III. N_{100}PK	2.08c	23.1d	2.21c	28.4c	38.6a
IV. N_{150}PK	2.38b	27.3c	2.34b	33.1bc	35.6ab
V. N_{200}PK	2.45b	27.6c	2.44a	37.0ab	33.5ab
VI. N_{250}PK	2.83a	28.8bc	2.44a	36.9ab	26.9bc
VII. N_{250}PK+PG	2.81a	33.4a	2.50a	36.1ab	26.9bc
VIII. N_{250}PK+D	2.84a	34.5a	2.52a	40.3a	31.6abc
IX. N_{300}PK	2.81a	31.9ab	2.49a	36.2ab	22.4c
Cultivar/Year					
“Fiesta”/2008	2.57a	30.6a	2.47a	23.8b	32.2a
“Lucija”/2010	2.16b	21.0b	2.25b	36.0a	29.4a
Growth stage					
F8	2.74a	33.3a	-	-	-
F10.5	1.99b	18.4b	-	-	-

[1]Means followed by the same letter within a column for each treatment, cultivar/year and growth stage are not significantly different at the $p \leq 0.05$ level by Duncan’s Multiple Range test

Analysis of variance was performed for all possible effects including each cultivar/year, each growth stage and each N fertilization treatment, and then for pooled effect of all factors on winter wheat variables: leaf nitrogen (N) content, chlorophyll content index (CCI), grain N content, yield, NUE and spectral parameters calculated as NDVI and RVI (Table 5.1.5 – 5.1.8). All investigated agronomic and spectral variables were strongly dependent on N application levels and increased with increasing N rates. The effect of N treatments on vegetation indices was statistically significant ($p < 0.001$).

Table 5.1.5. Analysis of variance for the leaf N content and CCI for each cultivar/year.

Cultivar/Year	Results of ANOVA[1]	Leaf TN %	CCI
„Fiesta“/2008	CV	14.39	19.30
	R^2	0.85	0.83
	Model	18.41***	15.09***
	Error	0.14	34.91
	GS	154.97***	128.48***
	Treatment	17.80***	15.11***
	Treatment x GS	1.95ns	0.90ns
„Lucija“/2010	CV	14.12	25.01
	R^2	0.83	0.86
	Model	15.29***	19.36***
	Error	0.09	27.67
	GS	30.88***	129.01***
	Treatment	27.21***	21.49***
	Treatment x GS	1.43ns	3.52**

[1]Are given MSE and F-values for the sources of variation considered; ns: not significant, level of statistical significance: ***p < 0.001; **p < 0.01; *p < 0.05

Table 5.1.6. Analysis of variance for NDVI and RVI for cultivar “Lucija”, 2010.

Cultivar/Year	Results of ANOVA[1]	NDVI – F8	RVI – F8
„Lucija“/2010	CV	5.81	10.88
	R^2	0.93	0.88
	Model	42.59***	24.01***
	Error	0.001	0.21

[1]Are given MSE and F-values for the sources of variation considered; ns: not significant, level of statistical significance: ***p < 0.001; **p < 0.01; *p < 0.05

Growing conditions and crop genetic properties significantly affected harvest variables, leaf TN content and CCI for each growth stage (Table 5.1.7).

Table 5.1.7. Analysis of variance for the leaf N content and CCI for each growth stage, and grain N content, yield and NUE for overall effect.

Growth stage	Results of ANOVA[1]	Leaf TN %	CCI		Results of ANOVA	Grain TN %	Yield $dtha^{-1}$	NUE %
F8	CV	14.11	15.13					
	R^2	0.83	0.86					
	Model	15.84***	19.99***					
	Error	0.15	25.36					
	Cultivar/Year	69.03***	77.27***	Yield variables	CV	3.81	15.75	32.13
	Treatment	23.86***	31.21***		R^2	0.85	0.91	0.33
	Treatment x Cultivar/Year	1.17ns	1.61ns		Model	18.01***	30.29***	1.58ns
					Error	0.01	22.22	97.76
F10.5	CV	14.25	33.24		Cultivar/Year	100.86***	120.93***	1.14ns
	R^2	0.75	0.70		Treatment	23.19***	46.99***	2.65*
	Model	9.57***	7.47***		Treatment x Cultivar/Year	2.47*	2.26*	0.59ns
	Error	0.08	37.22					
	Cultivar/Year	1.08ns	36.88***					
	Treatment	17.09***	9.51***					
	Treatment x Cultivar/Year	3.11**	1.75ns					

[1]Are given MSE and F-values for the sources of variation considered; ns: not significant, level of statistical significance: ***p < 0.001; **p < 0.01; *p < 0.05

When analyzing overall effect for laboratory and field measured variables acquired at F8 and F10.5 stages, the significant treatment x GS interaction was recorded due to wide range of variable responses (Table 5.1.8). Cultivars responded similarly to N fertilization levels at both growth stages according to ANOVA calculated for overall effect.

Table 5.1.8. Analysis of variance for the leaf N content and CCI for overall effect.

Results of ANOVA[1]	Leaf TN %	CCI
CV	14.33	21.66
R^2	0.85	0.86
Model	18.18***	19.51***
Error	0.11	31.29
GS	172.70***	256.59***
Treatment	40.30***	33.91***
Cultivar/Year	53.39***	105.66***
Treatment x GS	2.67**	2.70**
Cultivar/Year x GS	36.92***	0.83ns
Treatment x Cultivar/Year	2.90**	1.95ns
Treatment x Cultivar/Year x GS	0.80ns	1.43ns

[1]Are given MSE and F-values for the sources of variation considered; ns: not significant, level of statistical significance: ***p < 0.001; **p < 0.01; *p < 0.05

5.2 Visual evaluation of spectral data

Spectral data were visually evaluated before and after pre-treatment. Pre-processing methods included normalizing to minimize the variations due to illumination intensity changes and distance between the leaf and fiber optic probe. Figure 5.2.1a, 5.2.1b shows winter wheat leaf reflectance spectra (a) and its first derivative (b). Mean spectral data of nine fertilization levels were grouped into three classes in which the means were statistically different from one another [I (Control, N_0); II (N_{100}, N_{150}, N_{200}); III (N_{250}, N_{300}) (kg ha^{-1})]. The greatest spectral differences between treatment classes were in the green to red and at the red edge region. Compared to well fertilized group of treatments, nitrogen limitation in group I highly increased red reflectance and decreased NIR reflectance.

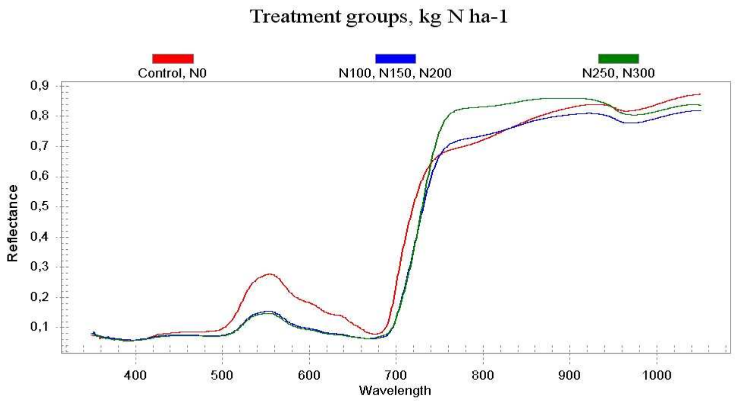


Figure 5.2.1a – Average raw reflectance factor for winter wheat flag leaves for three groups of different N fertilizer levels acquired on May 07, 2010 (F8 - "Lucija") (n=36).

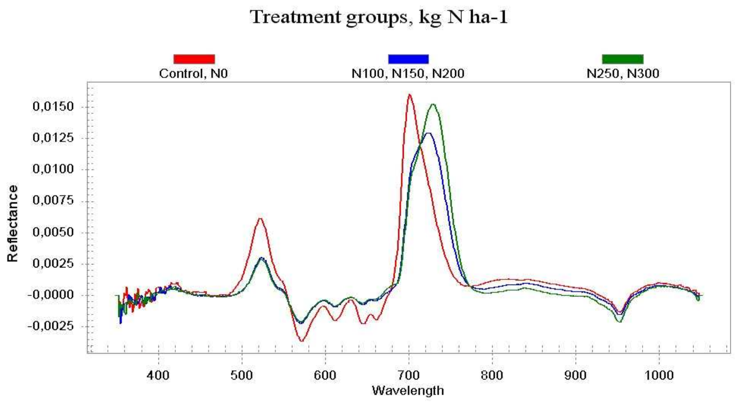


Figure 5.2.1b – Average first derivative reflectance factor for winter wheat flag leaves for three groups of different N fertilizer levels acquired on May 07, 2010 (F8 - "Lucija") (n=36).

5.3 Correlation analysis between spectral data and crop variables

Correlation coefficients between the leaf nitrogen (N) content, chlorophyll content index (CCI), grain N content, yield, NUE and spectral parameters calculated as NDVI and RVI are summarized in Table 5.3.1. Reported data refer to stem extension stage (F8) for leaf measurements and vegetation year 2010 (cultivar "Lucija") for harvest results. Very strong to full significant correlations were found between leaf TN content, CCI, grain TN content, yield, and both vegetation indices. Correlogram between winter wheat grain yield and leaf spectra (single wavelength) in the form of raw reflectance (raw), first derivative (dv1) and second derivative (dv2) of reflectance acquired at the same time is shown in Figure 5.3.1. Dash lines on the graph represent the significant correlations at $p < 0.05$. The first derivative transformation of spectra showed higher overall *r* values than the corresponding raw reflectance. Significantly high correlations with grain yield for both reflectance and two derivatives were obtained in the visible and red edge part of spectrum indicating chlorophyll optical properties.

Table 5.3.1. Correlation coefficients among winter wheat variables and spectral data measured during growth stage F8 and harvest, 2010 – "Lucija" (n = 36).

Variable	Means	SD	Leaf TN, %	CCI	Grain TN, %	Yield, $dtha^{-1}$	NDVI	RVI
Leaf TN, %	2.359	0.696		0.922	0.785	0.775	0.802	0.787
CCI	28.072	12.204	0.922[1]		0.744	0.798	0.827	0.821
Grain TN, %	2.253	0.210	0.785	0.744		0.752	0.748	0.758
Yield, $dtha^{-1}$	36.033	11.247	0.775	0.798	0.752		0.905	0.872
NDVI	0.593	0.112	0.802	0.827	0.748	0.905		0.986
RVI	4.220	1.149	0.787	0.821	0.758	0.872	0.986	

[1]Marked correlations are significant at $p < 0.05$

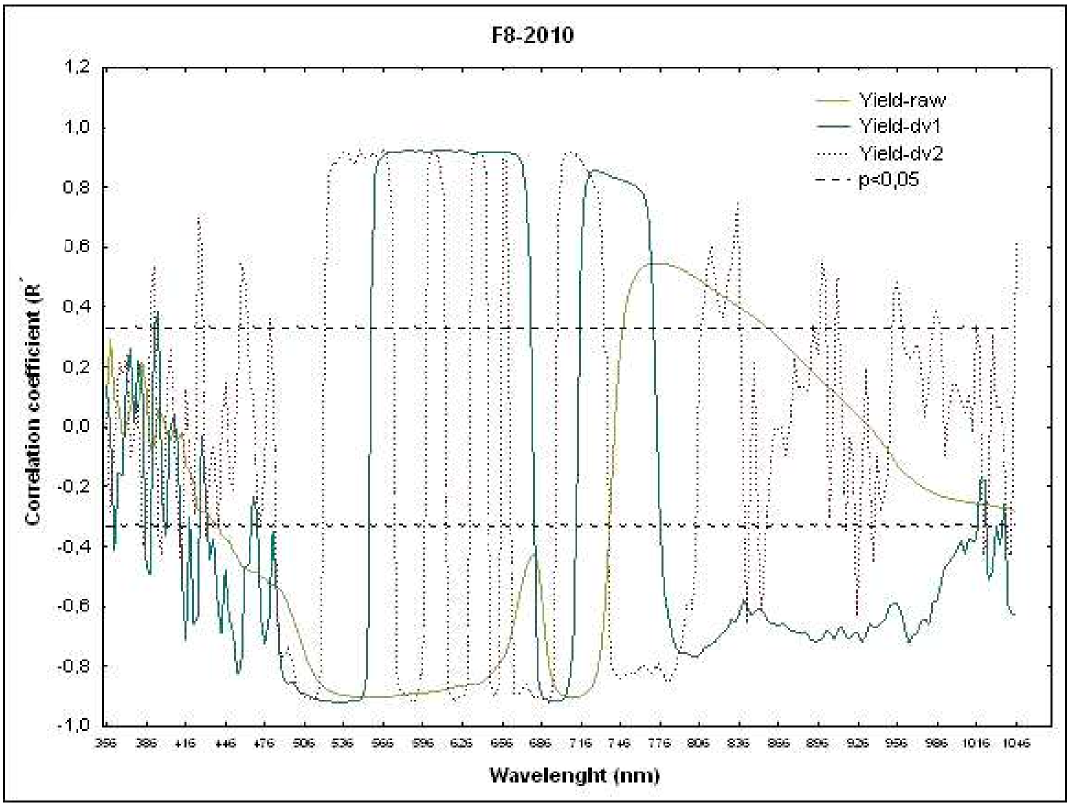


Figure 5.3.1 – Correlogram showing relationship between winter wheat yield and leaf raw spectral reflectance, first derivative (dv1) and second derivative (dv2) of reflectance acquired by the field spectroradiometer on May 07, 2010 (F8 – stem extension, "Lucija"/2010).

Figures 5.3.2 and 5.3.3 show 1:1 relationships between winter wheat yield and vegetation indices NDVI and RVI calculated from raw reflectance data measured at leaf level during stem extension (F8) of vegetation period 2010 with cultivar "Lucija". Dash lines on the graph represent the significant relationships at $p < 0.05$. Very strong positive and significant relationship was achieved between narrow band spectral indices and winter wheat yield. Significant but medium positive correlations were found between NDVI and RVI and winter wheat yield at heading growth stage (F10.5–2010-"Lucija") ($p < 0.05$) (Figure 5.3.4; Figure 5.3.5).

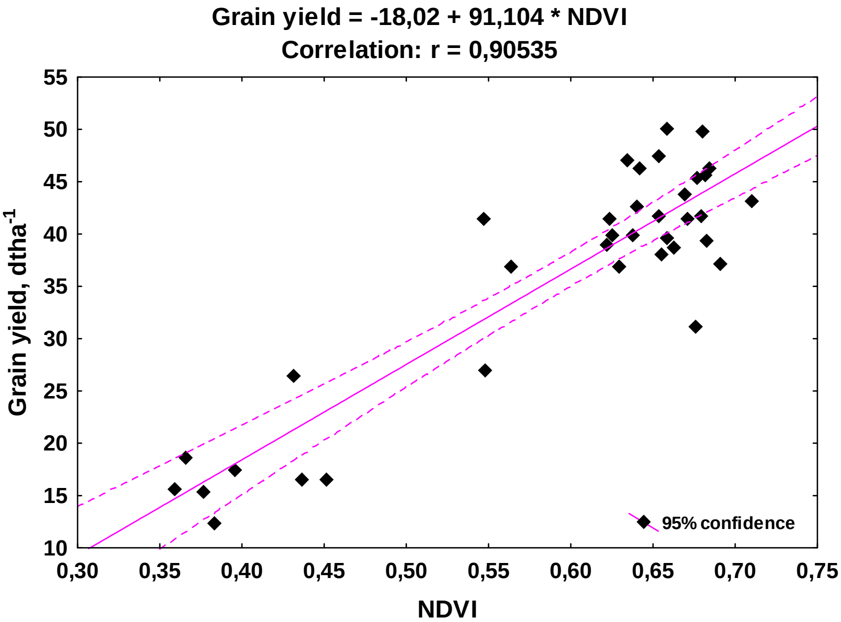


Figure 5.3.2 – The relationship between winter wheat yield and NDVI developed from raw reflectance data acquired at leaf level during growth stage F8 - "Lucija"/2010 ($p < 0.05$; total number of samples = 36).

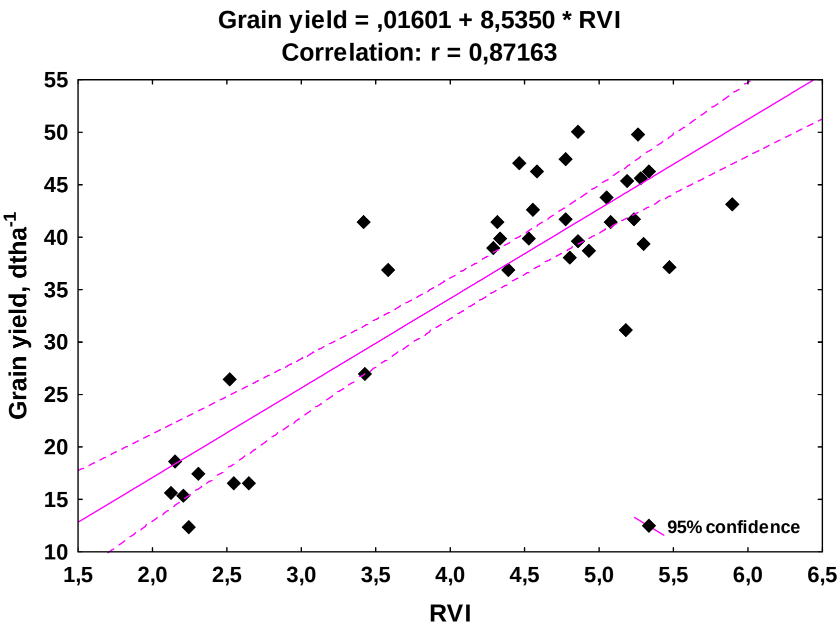


Figure 5.3.3 – The relationship between winter wheat yield and RVI developed from raw reflectance data acquired at leaf level during growth stage F8 - "Lucija"/2010 ($p < 0.05$; total number of samples = 36).

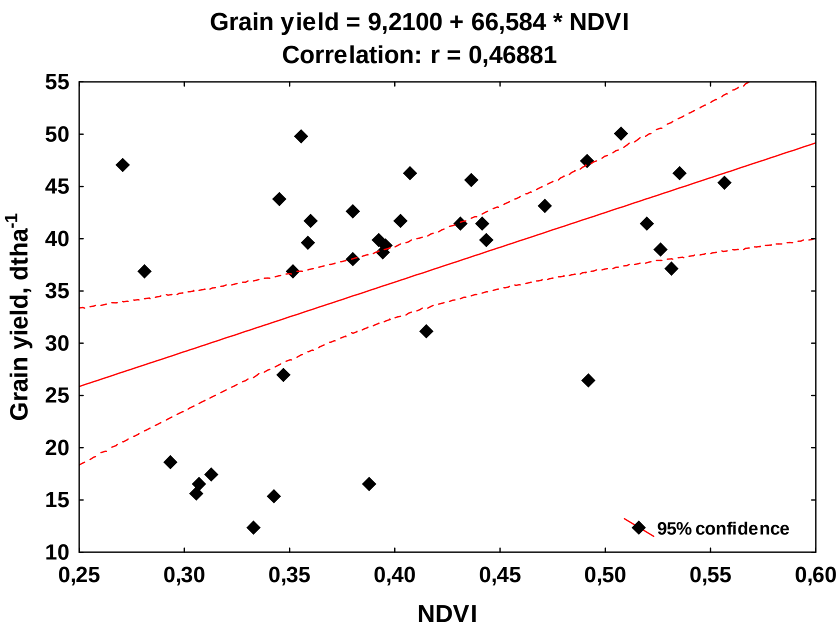


Figure 5.3.4 – The relationship between winter wheat yield and NDVI developed from raw reflectance data acquired at leaf level during growth stage F10.5 - "Lucija"/2010 ($p < 0.05$; total number of samples = 36).

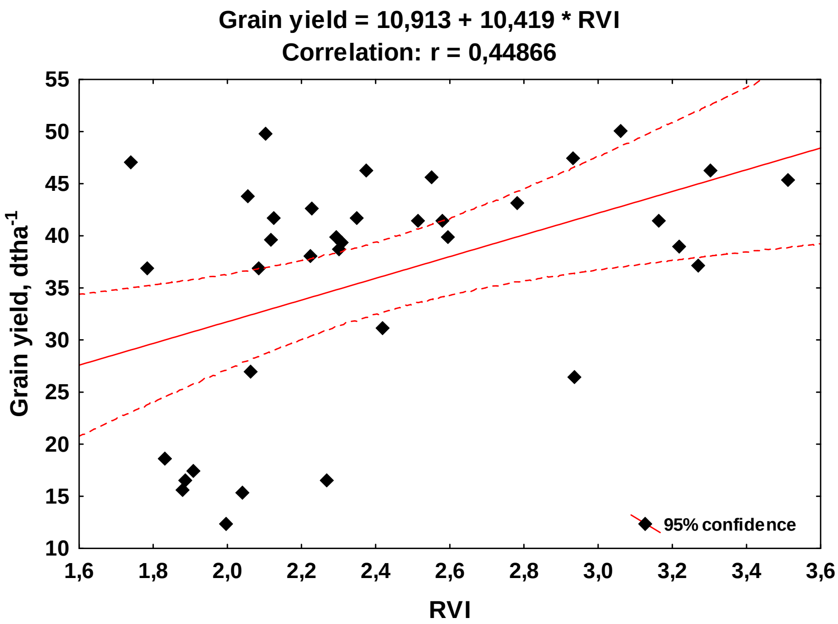


Figure 5.3.5 – The relationship between winter wheat yield and RVI developed from raw reflectance data acquired at leaf level during growth stage F10.5 - "Lucija"/2010 ($p < 0.05$; total number of samples = 36).

5.4 Multivariate linear and non-linear modeling – regression

To better understand the use of field spectroscopy in monitoring of crop nutrient status, Figure 5.4.1 was built to illustrate simplified scheme of all activities used to estimate winter wheat variables from spectral data, including field measurements, laboratory analysis, fitting the model for calibration as well as full cross-validation by applying spectral data on the equation.

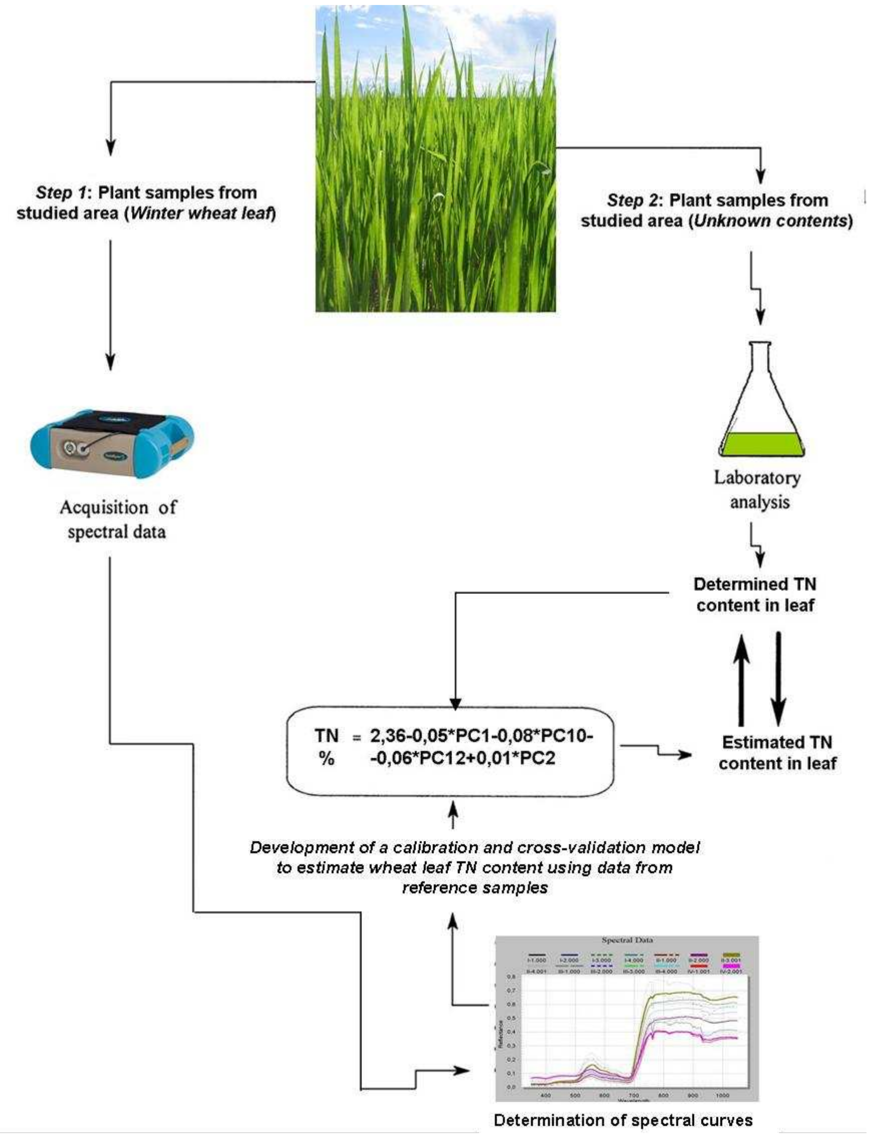


Figure 5.4.1 – Illustration of the modeling activities flow – example of TN content in winter wheat leaf, F8-2010.

5.4.1 Simple linear regression (SLR), multiple linear regression (MLR) and partial least squares regression (PLSR)

Results of the multiple linear regression (MLR) model calculated using spectral data (PCs) and winter wheat yield data are shown in Figure 5.4.1.1 Upper two plots show graphically the relationship between the predicted and measured grain yield in the calibration and the validation sets. Residual vs. predicted yield graph explained deviations between the observed data values and the model approximation of those values. Line plot of regression coefficients (right below) checked the importance of the different X-variables (spectra) in predicting yield. The regression coefficients for 7 PCs summarized the relationship between the predictors and the response, as a model with 7 components approximated it. Model adequately predicted variations in grain yield with very strong dependence among variables (R^2 = 0.89 and R^2 = 0.83 for calibration and validation, respectively).

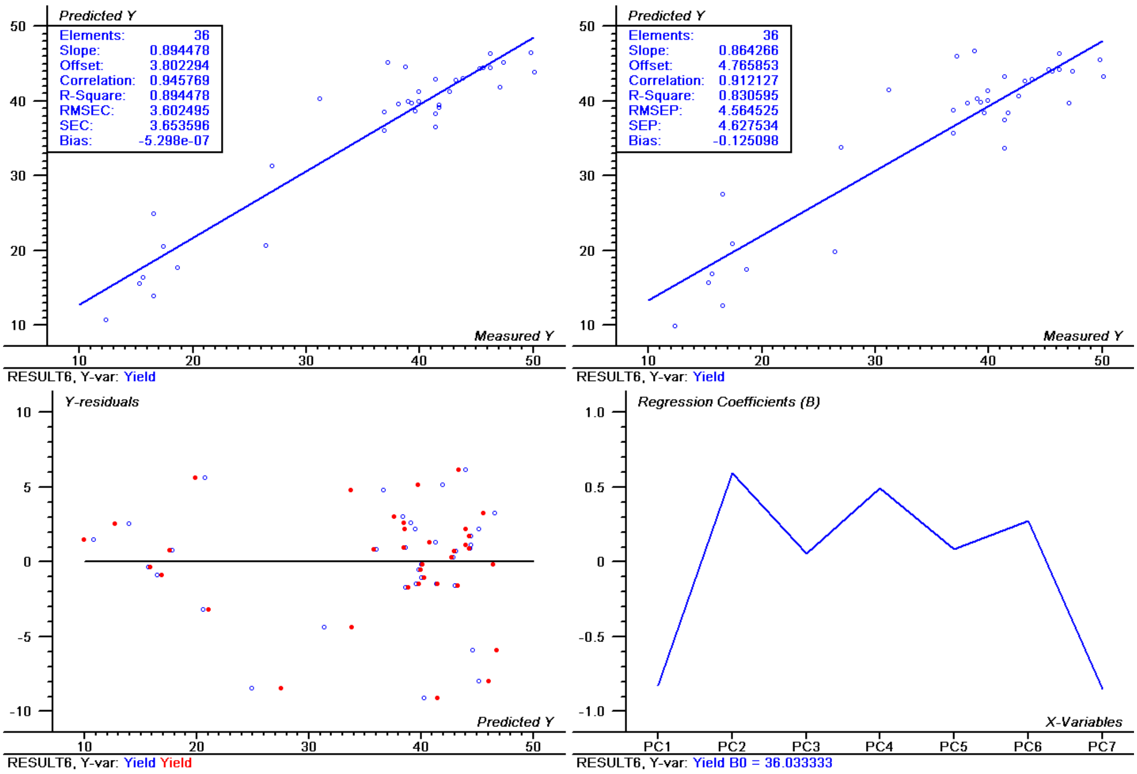


Figure 5.4.1.1 – Scatter plots for the results of the MLR model performed using full cross validation method from 1st derivative of reflectance acquired at growth stage F8- "Lucija"/2010 showing relationship between predicted and observed winter wheat yield (dt ha^{-1}) (n=36) ● calibration ● validation.

Figure 5.4.1.2 shows scatter plots with calibration and full cross validation results of the MLR model for predicted (Y axis) and observed (X axis) leaf TN content, CCI and grain TN content. Five principal components of the 1st derivative of reflectance acquired at growth stage F8 of cultivar "Lucija" during 2010 were used as independent (predictor) input data for all observed variables in the model. Grain TN content estimation model had the highest coefficient of determination (R^2 = 0.85), followed by CCI (R^2 = 0.72) and leaf TN content (R^2 = 0.69). The RMSEP were 0.383 %, 6.392 and 0.08 % for leaf TN content, CCI and grain TN content, respectively.

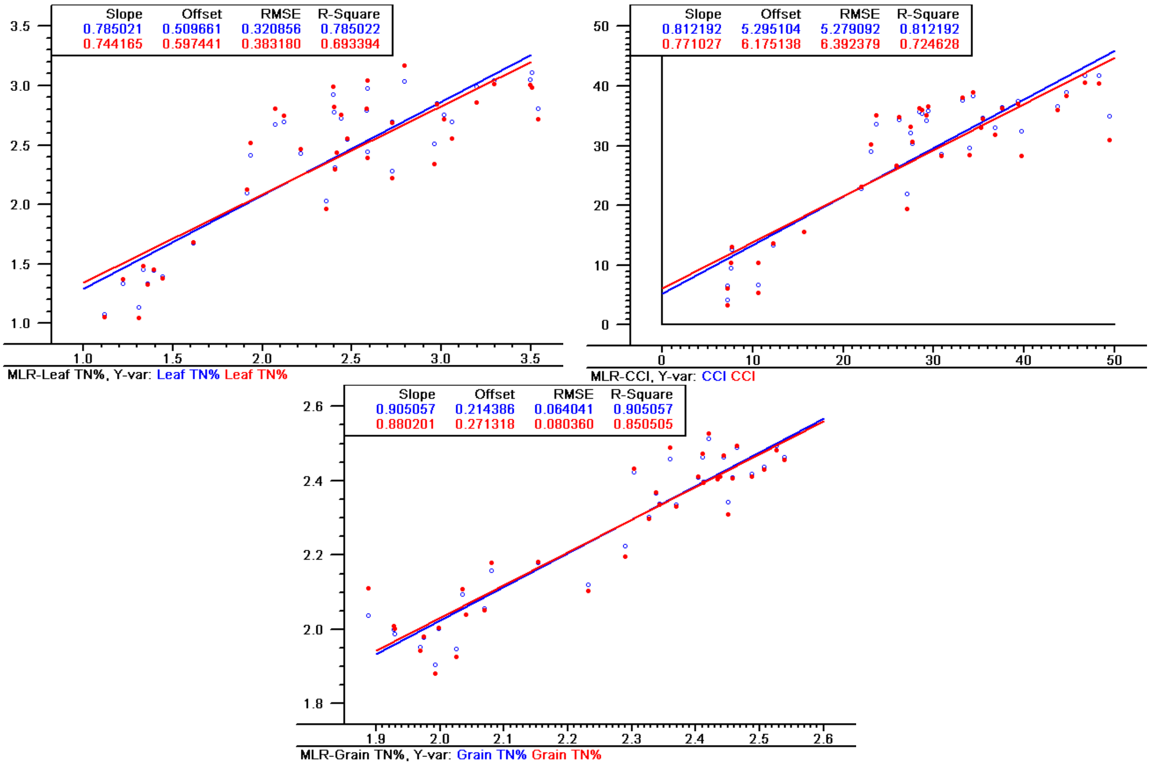


Figure 5.4.1.2 – Scatter plots of the MLR model performed using full cross validation method from 1st derivative of reflectance acquired at growth stage F8 - "Lucija"/2010 showing relationship between predicted and observed leaf TN content (%), CCI and grain TN content (%) (n=36) ● calibration ● validation.

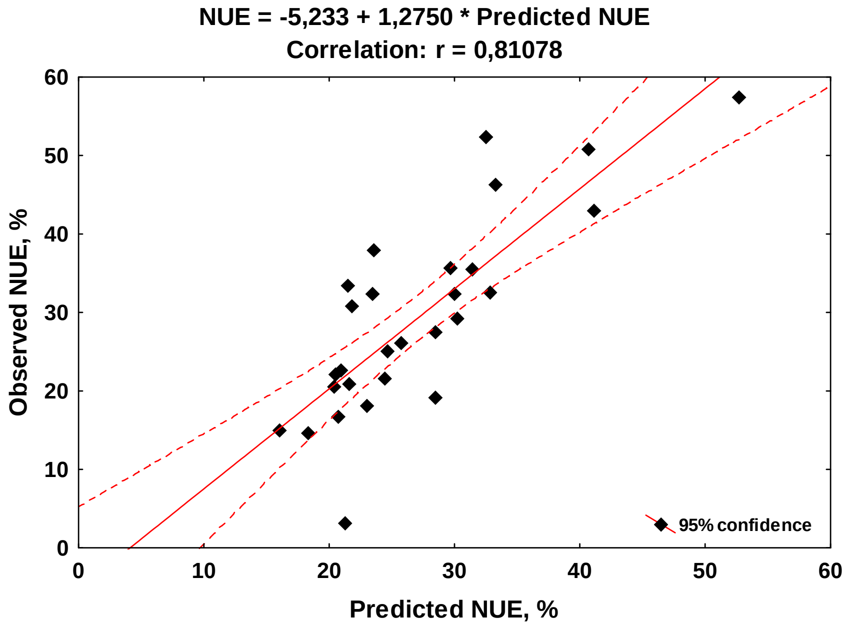


Figure 5.4.1.3 – Comparison between PLSR model predicted and field observed nitrogen use efficiency (NUE, %) (n=36).

1:1 relationship between the predicted and observed nitrogen use efficiency in winter wheat (NUE %) is shown as a scatter plot on Figure 5.4.1.3. As agronomic indicator, NUE was calculated based on harvest data of "Lucija" cultivar during vegetation period 2010: grain TN content, yield in form of N uptake, and amount of nitrogen applied. Predicted NUE was calculated based on the PLSR full cross validation results for harvest data, and then regressed against the observed values. Very strong significant relationship was found between observed and predicted NUE (r = 0.81).

Figure 5.4.1.4 and 5.4.1.5 show results of the partial least square regression (PLSR) model calibrated to estimate winter wheat yield using first derivative of reflectance (231 wavelength across full spectral range of 350-1050 nm) and yield data (36 samples). Eight PCs were chosen to represent the main structured information in the spectral dataset. Upper two plots represent a two dimensional scatter plot of scores and loadings for two specified components from PLSR. The score plot gave information about patterns in the samples. First two components summarized the most variation in the data (PC1: 69 % and PC2: 13 %). Loadings for spectral and yield data showed contributions of each variable to the meaningful variation in the data, and how well that PC takes into account the variation of yield variable over the data points.

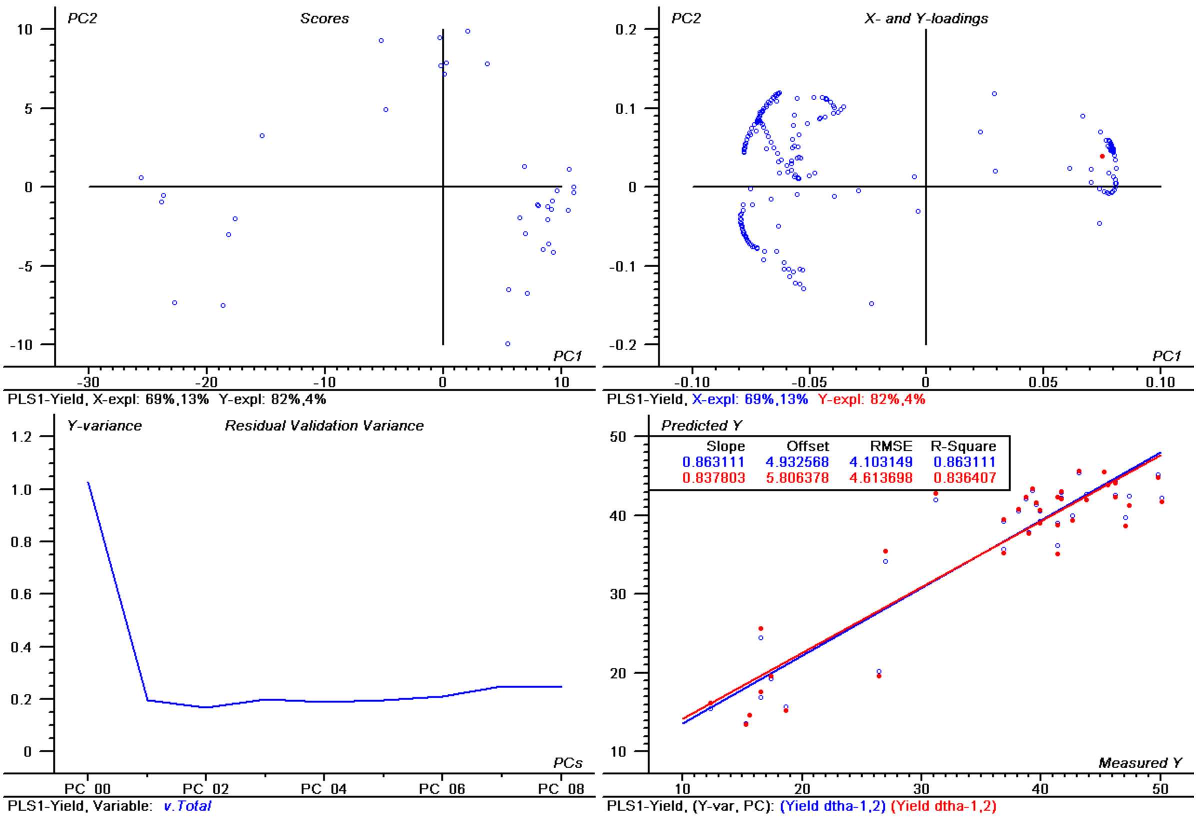


Figure 5.4.1.4 – Scatter plots for the results of the PLSR model performed using full cross validation method from 1st derivative of reflectance acquired at growth stage F8 - "Lucija"/2010 showing relationship between predicted and observed winter wheat yield (dt ha^{-1}) (n=36) ● calibration ● validation.

Residual validation variance expressed how much variation in the yield data remains to be explained once the current spectral PCs have been taken into account. Full correlation between the predicted and measured grain yield in the calibration and the validation datasets (r = 0.93 and r = 0.91, respectively) are shown in the scatter plot right below (Figure 5.4.1.4). Figure 5.4.1.5 shows a line plot of X-loading weights for two specified components from a PLSR analysis important for detecting which spectral variables are most significant for predicting winter wheat yield. Visible part of spectrum (550-670 nm and 690-710 nm) with red edge region (730-770 nm) were identified as zones of major importance for the PLSR model of winter wheat yield. Residual vs. predicted yield graph (below) explains deviations between the observed data values and the model approximation of those values.

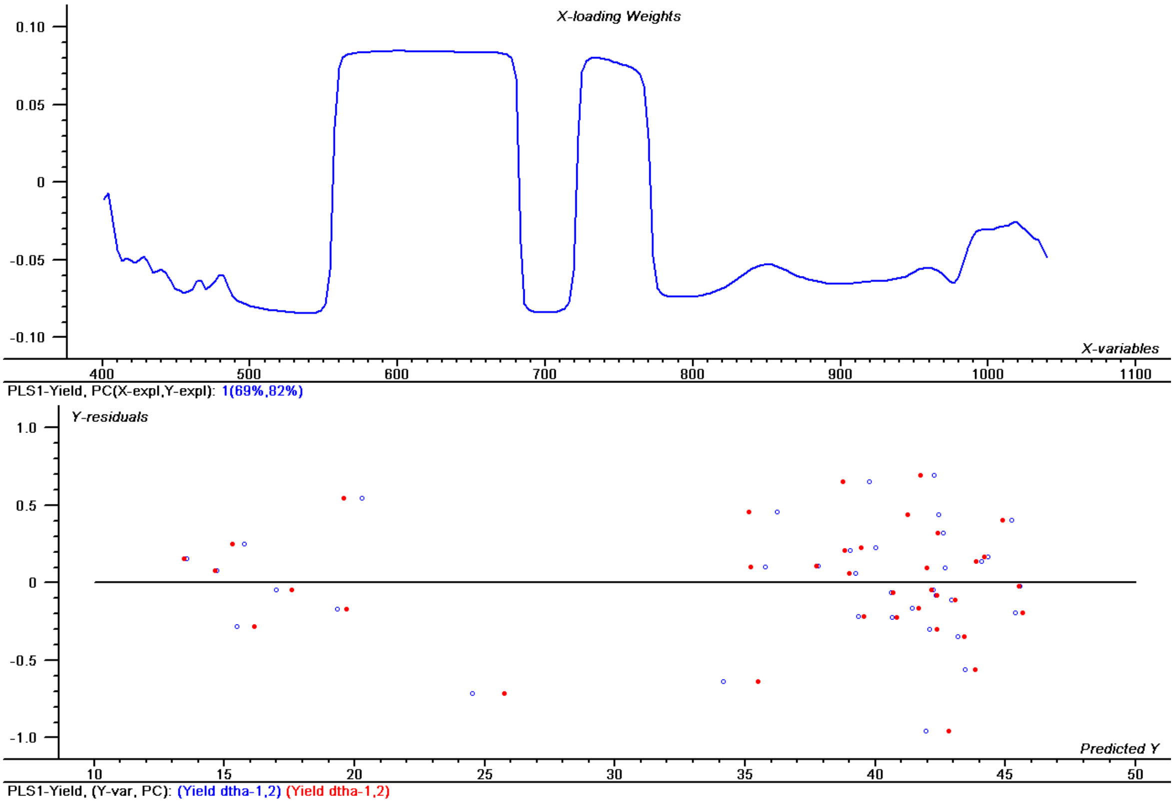


Figure 5.4.1.5 – Loading weights (a) and residuals (b) for the PLSR models calibrated to estimate winter wheat yield. High numerical values of regression coefficients indicate high importance of the spectral band in the PLS analysis. Suggested number of components is 2.

● calibration ● validation

5.4.2 Artificial neural networks (ANN) - regression

Predicted vs. observed winter wheat yield relationship for train (calibration) and test (validation) dataset explained using ANN regression modeling was found to have full correlation: r = 0.95 and r = 0.92, respectively (Figure 5.4.2.1). Pattern recognition and prediction of yield from spectral data was performed using PC scores of 1st derivative of reflectance as input neurons (total of 16 PCs). Dash lines on the graph represent the significant relationships at $p < 0.05$.

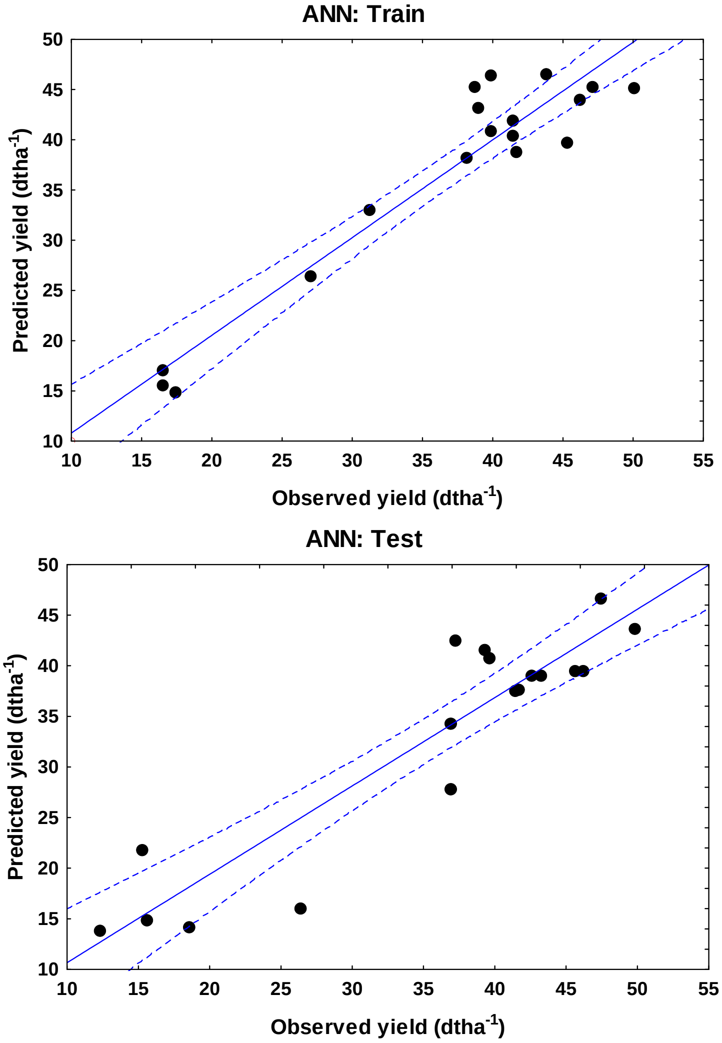


Figure 5.4.2.1 – Comparison of predicted and observed winter wheat yield using artificial neural network model for regression task. Training set: 50%, Testing set: 50% of samples. Input neurons are PCA scores of 1st derivative of reflectance, F8 - "Lucija"/2010.

Neural network structure and internal parameters are summarized in Table 5.4.2.1. Optimal back propagation ANN architecture was MLP (Multilayer perceptron) 16-9-1 with 1 hidden layer (16: input PCs; 9: number of hidden units or neurons; 1: output winter wheat yield) and BFGS iterative technique.

Table 5.4.2.1. Summary of optimum ANN regression model architecture and internal parameters (input neurons are PCA scores of 1st derivative of reflectance, F8 - "Lucija"/2010).

Index	Net. name	Training perf. (r)	Test perf. (r)	Training error	Test error	Training algorithm	Error function	Hidden activation	Output activation
52	MLP 16-9-1	0.950257	0.924875	0.004831	0.011774	BFGS 4	SOS	Tahn	Identity

Summary of multivariate regression and ANN models

Summary of statistical parameters for regression type of modeling between spectral data and crop variables is reported in Table 5.4.2.2. Performance and model accuracy analysis of NDVI and RVI in the form of SLR, then MLR, PLSR and ANN in predicting winter wheat yield is represented for calibration and validation. RMSE is expressed in original units of the response variable. ANN model gave the highest coefficients of determination (R^2) and the lowest root mean squared errors (RMSE) than the corresponding SLR-VIs, MLR and PLSR models, in both calibration and validation tests.

Table 5.4.2.2. Summary of calibration and validation results of SLR, MLR, PLSR and ANN models for grain yield of cultivar "Lucija" using VIs and PCs of spectra 1st derivative acquired during growth stage F8, 2010.

Model	NPC	Calibration		Full cross-validation (leave-1-out)	
		R^2	RMSEC (dt ha^{-1})	R^2	RMSEP (dt ha^{-1})
SLR - NDVI	-	0.820	4.710	0.800	4.954
SLR - RVI	-	0.760	5.436	0.732	5.745
MLR	7	0.894	3.602	0.831	4.565
PLSR	8	0.863	4.103	0.836	4.614
ANN	16	0.903	2.571	0.856	4.406

NPC: optimal number of principal components; R^2: coefficient of determination; RMSEC: root mean square error of calibration; RMSEP: root mean square error of prediction

The spatial variability of the winter wheat yield was mapped using ArcView geographic information system (GIS) (ESRI, 2006) for both measured and predicted values to visually express within-field and intra-treatment differences in crop productivity needed for assessing good calibration model. Ordinary kriging interpolation method was used for mapping. Figure 5.4.2.2 show the grain yield maps generated from the reference measurements and from estimates by relationships developed using linear and ANN modeling. Yield maps represent the F8 growth stage spectral measurements on cultivar "Lucija" flag leaves. ANN map was the closest to measured winter wheat yield considering spatially comparable estimates. Disagreements mostly occurred on treatments VI, VII and VIII due to the high variability in the yield data within the same treatments.

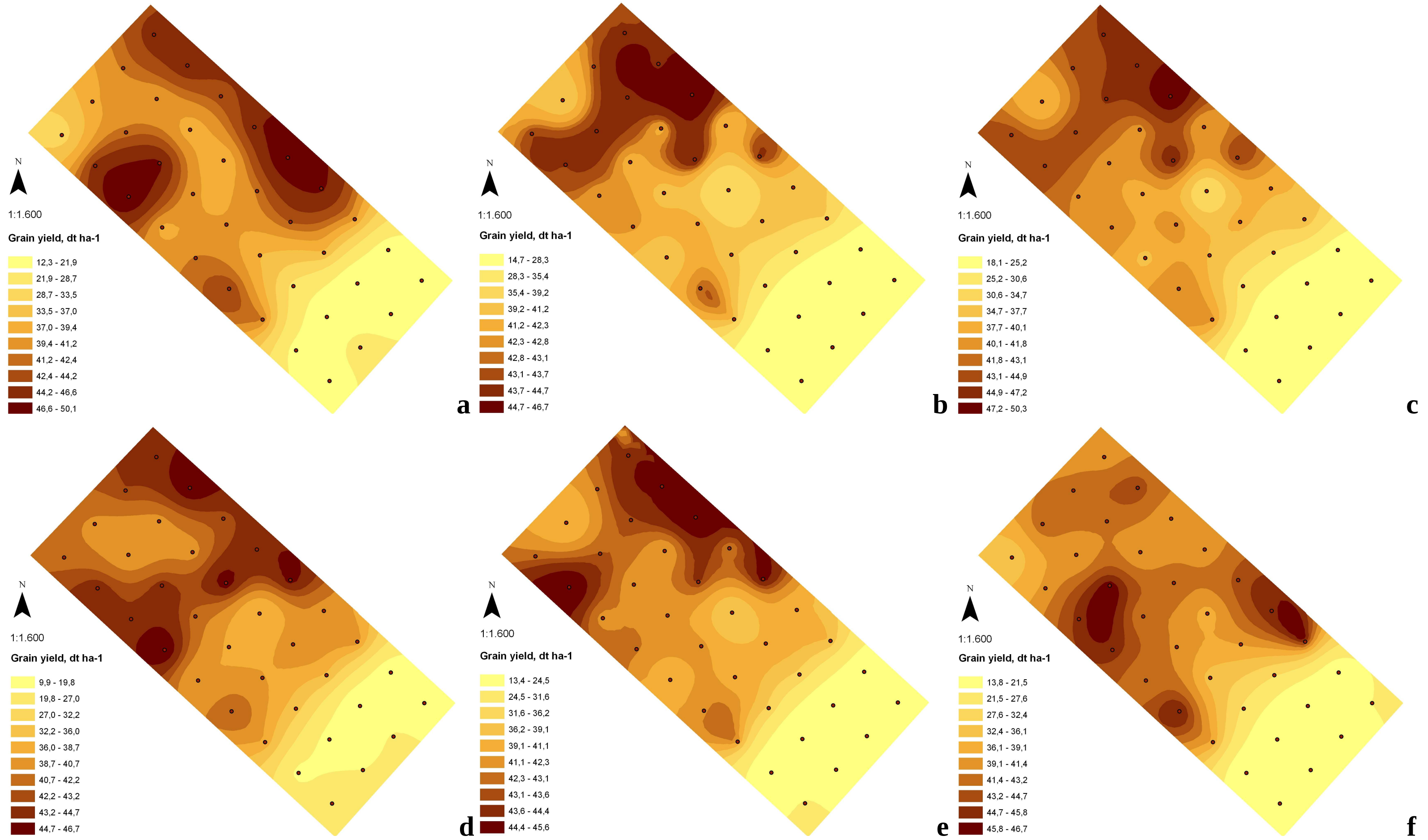


Figure 5.4.2.2 – Grain yield (dt ha^{-1}) maps for winter wheat cultivar “Lucija”: a) as measured; b) estimated by NDVI; c) estimated by RVI; d) estimated by MLR; e) estimated by PLSR; f) estimated by ANN. (estimations based on spectral data acquired at the stem extension growth stage - F8)

5.5 Classification analysis based on spectral signatures

5.5.1 Discriminant analysis

Discriminant analysis (DA) was used to predict the amount of N fertilizer rate applied to the winter wheat using spectral data. Summary of DA with statistical parameters important for the interpretation is presented in Table 5.5.1.1. The basic idea underlying DA was to determine whether groups of N fertilization levels differ with regard to the mean of a spectral component, and then to use that spectral PCs to predict group membership. Standard statistics reported in the table was used to denote the statistical significance of the discriminatory power of the current model and unique contribution of the respective spectral PC to the discrimination between N level groups. Values of the statistical parameters Wilks lambda and Partial Wilks lambda ranged between 0 (perfect discriminatory power) and 1 (no discriminatory power). Nine PCs were marked to have significant contribution to N levels discrimination (p<0.05).

Table 5.5.1.1. Discriminant Function Analysis Summary (PCA scores - 1st derivative of reflectance, F8 - "Lucija"/2010), Step 15, N of variables in model: 15; Grouping: Treatments (3 groups); Wilks Lambda: 0.00125 approx. F (30.38) = 34.547, p < 0,0000.

N=36	Wilks Lambda	Partial Lambda	F-remove (2,19)	p-level	Toler.	1-Toler. (R-Sqr.)
Component 1	0.084828[1]	0.014746	634.7222	0.000000	0.140128	0.859872
Component 2	0.008434	0.148317	54.5521	0.000000	0.223463	0.776537
Component 10	0.006062	0.206360	36.5361	0.000000	0.267981	0.732019
Component 3	0.003940	0.317511	20.4202	0.000018	0.397552	0.602448
Component 13	0.002924	0.427846	12.7042	0.000314	0.448277	0.551723
Component 5	0.001915	0.653095	5.0461	0.017469	0.668406	0.331594
Component 8	0.001918	0.652346	5.0628	0.017280	0.686642	0.313358
Component 14	0.001957	0.639188	5.3626	0.014239	0.655655	0.344345
Component 16	0.001777	0.703834	3.9975	0.035561	0.709095	0.290905
Component 4	0.001712	0.730537	3.5041	0.050652	0.753399	0.246601
Component 6	0.001614	0.774966	2.7586	0.088753	0.780141	0.219860
Component 11	0.001536	0.814503	2.1636	0.142390	0.820261	0.179739
Component 15	0.001458	0.858169	1.5701	0.233852	0.864018	0.135982
Component 7	0.001473	0.849362	1.6849	0.212021	0.863190	0.136810
Component 12	0.001466	0.853402	1.6319	0.221799	0.867100	0.132900

[1]Marked correlations are significant at p < 0.05

Table 5.5.1.2 shows parameters that explain significance of the two selected discriminant functions (roots). The first function accounted for over 99 % of the explained variance.

Table 5.5.1.2. Chi-Square Tests with Successive Roots Removed (PCA scores - 1st derivative of reflectance, F8 - “Lucija”/2010).

Roots removed	Eigenvalue	Canonical R	Wilks Lambda	Chi-Sqr.	df	p-level
0	73.72191	0.993286	0.001251	173.7810	30	0.000000
1	9.69857	0.952118	0.093470	61.6229	14	0.000000

Figure 5.5.1.1 graphically represents discrimination between three groups of N fertilization levels based on spectral data of winter wheat flag leaves. It is a scatterplot of canonical scores for the two statistically significant discriminant functions. Clearly, the winter wheat leaves under N treatments of group I are plotted much further to the right in the scatterplot. Thus, the first discriminant function mostly discriminated between group I and the other two. The second function showed discrimination between the group II (which mostly show positive values for the second canonical function) and the others (which have mostly negative values).

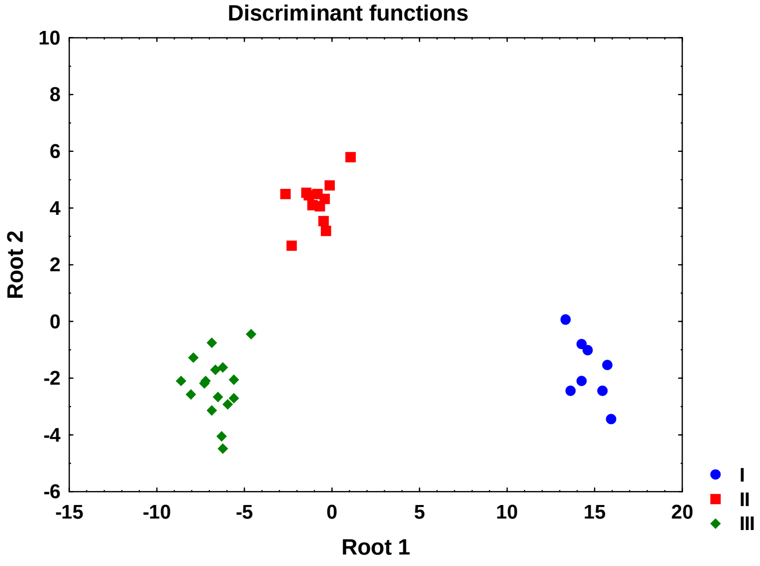


Figure 5.5.1.1 – Sampled winter wheat leaves represented as function of discriminant factors (roots) 1 and 2 based on the PCA scores of 1st derivative of reflectance, F8 - “Lucija”/2010. Samples are labeled according to the N treatment group: I (Control, N_0); II (N_{100}, N_{150}, N_{200}); III (N_{250}, N_{300}) (kg ha^{-1}).

5.5.2 Clustering

Clusters of the statistically different groups of N fertilization levels based on the first derivative of the reflectance are plotted in Figure 5.5.2.1. The spectral parameters in the form of PCs were grouped into K- (3) clusters based on a specific distance measurement, so that the sum of distances between each sample and its cluster centroid was minimized. The upper two plots represent a two dimensional scatter plot of scores and loadings for two PCs. Score scatterplot showed almost completely discrimination between treatment groups 1 (N_{250}, N_{300} kg ha^{-1}), 2 (N_{100}, N_{150}, N_{200} kg ha^{-1}) and 3 (Control, N_0 kg ha^{-1}). The spectra loadings were comparable to score plot, indicating correlation and importance of each spectral feature for differing N levels. Influence plot showed which variables had a high influence on the model so that it describes them better, like II-1, II-3 and V-2. Sample I-1 seemed to be an outlier. Explained variance showed the proportion of variation in the data accounted for by the current PCs.

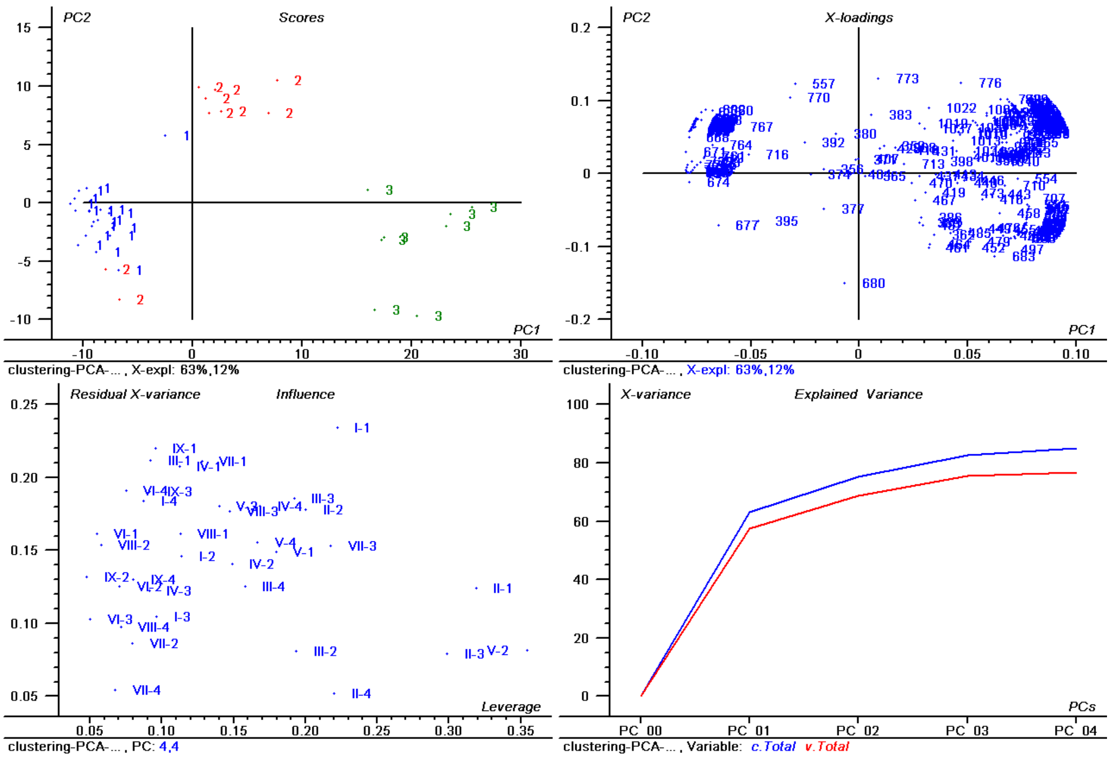


Figure 5.5.2.1 – Cluster analysis of three statistically different groups of N fertilizer treatments (3-Control, N_0; 2-N_{100}, N_{150}, N_{200}; 1-N_{250}, N_{300}) using PCA scores of 1st derivative of leaf reflectance, F8 - "Lucija"/2010.

5.5.3 Artificial neural networks (ANN) - classification

Neural networks were also used to tackle classification problem of differing N fertilizer levels based on leaf spectral reflectance. The optimum ANN classification model architecture and internal parameters are reported in Table 5.5.3.1. Optimal back propagation ANN architecture was MLP (Multilayer perceptron) 16-9-3 with 1 hidden layer (16: input PCs; 9: number of hidden units or neurons; 3: output of three N fertilizer level categories/groups) and BFGS iterative technique. By contrast to regression problems, a neural network classifier in this case assigned class membership to a three input categories. Classification summary is given in Table 5.5.3.2. One variable was misclassified in group II representing group III indicating 91 % statistically correct classified variables in the intermediate treatment group II.

Table 5.5.3.1. Summary of optimum ANN classification model architecture and internal parameters (input neurons are PCA scores of 1st derivative of reflectance, F8 - “Lucija”/2010).

Index	Net. name	Training performance (r)	Test performance (r)	Training algorithm	Error function	Hidden activation	Output activation
7	MLP 16-9-3	100.0000	94.44444	BFGS 13	Entropy	Identity	Softmax

Table 5.5.3.2. Prediction accuracy of fertilizer treatment group (I, II, III) classification using artificial neural networks - Classification summary (PCA scores of 1st derivative of reflectance, F8 - “Lucija”/2010); Samples: Train, Test.

	I (Control, N_0)	II (N_{100}, N_{150}, N_{200})	III (N_{250}, N_{300})
Total	8	12	16
Correct	8	11	16
Incorrect	0	1	0
Correct (%)	100	91	100
Incorrect (%)	0	8	0

6 DISCUSSION

Statistical analyses of differences in leaf TN and CCI, grain TN, NUE, yield and two vegetation indices (NDVI and RVI) according to fertilization treatments for each growth stage and cultivar/year were computed by analysis of variance (ANOVA). The significance test for overall statistics was performed at probability level of $p < 0.05$.

The wide range in field measured and laboratory analyzed winter wheat variables resulted from a large variation in nitrogen fertilization levels (0 – 300 kg N ha^{-1}) as defined by experiment methodology, vegetation years and cultivars (2 years; 2 cultivars – one each year) and sampling dates (2 growth stages per year) (Table 5.1.1). This was particular well-designed experiment situation in order to make relation between winter wheat growth, N status, and yield and leaf reflectance as realistic and universal as possible. When interpreting data pooled for all experiment factor combination, the absolute range of analyzed variables varied widely. This was also result of different climatic conditions per each year (Figure 4.1.3; Figure 4.1.4) and unfavorable soil properties (Figure 4.1.7). When analyzing fertilization treatments pattern, flag leaf TN content varied across cultivars/years and growth stages (Figure 5.1.1; Figure 5.1.2). It was higher during stem extension (F8) stage 2008 compared to the same growth stage at 2010. This can be explained by extreme climatic conditions during crop development. 2008 was problematic year in terms of lack of precipitation at critical growth stages of wheat (May, Figure 4.1.3) and monthly temperatures higher than average during stem extension and heading (Figure 4.1.4). 2010 was, as opposite, unusually wet compared to the average values, but still warmer than average (May and June, Figure 4.1.3). Rainfall amount doubled during summer months and its inter-annual path showed unusual variability compared to the reference period. Soil water balance for two extreme years (2008 and 2010) showed large difference in seasonal pattern of water surplus and water deficit (Figure 4.1.5). Beside water deficit during spring and summer months in 2008, the problem was also found in insufficient water supply during winter period needed for forthcoming intensive evapotranspiration caused by temperatures much higher then average. Majority of the adverse climate impact on crop growth in 2008 was caused by water shortages during critical phenophases, followed by surface water stagnation during 2010. Higher TN concentrations during 2008 with cultivar “Fiesta” can be explained by efficient plant uptake, dry conditions and reduced nitrogen leaching (Kastori, 2005). Differences in leaf TN content accounted for heading stage between cultivars and years were negligible (Figure 5.1.1) because N movement from leaves to grain was not affected by unfavorable climatic conditions. Grain TN content followed the same trend when comparing two vegetation years (Figure 5.1.5). Average concentration was statistically lower in 2010 (2.25 %) compared to 2008 (2.47 %) (Table 5.1.4). It was found that cultivar “Fiesta” (2008) had higher mean values of CCI for both stages than cultivar “Lucija” which can be explained by unfavorable climate properties expressed by water surplus and lower crop development in 2010 (Figure 5.1.4). However, pattern of differences between stem extension (F8) and heading (F10.5) was the same for both cultivars/years: CCI readings were statistically higher for F8 then for F10.5 stage (Table 5.1.4). Both investigated years were generally unfavorable for achieving optimal winter wheat yield potential, including synergy of the other environmental conditions except climate and water stress, like reduced soil pH after long-year period of fertilization on Stagnosol, soil compaction due to extreme precipitations, water retention due to lack of subsoiling and problems with canopy destruction by wildlife from Park of Nature Lonjsko polje (for 2008). The average yield for both cultivars and growing periods and across all N fertilization treatments was only 29.2 dt ha^{-1}, which was 34.5 % lower compared to the national average (45.8 dt ha^{-1}) for the last decade (2000-2009) (DZS, 2010). However, when consider all external effects, the expected influence of N fertilization on agronomic variables was recorded which was important for evaluation of spectral data and modeling. Nitrogen was a key factor which defined level of winter wheat yield. Grain yield had in both years significantly increasing trend with higher N fertilization levels, but it was lower in 2008 then in 2010 due to the soil water deficit and mentioned canopy damages. The maximal average yield per treatment was 44.7 dt ha^{-1} (2010 – cultivar „Lucija“) for N_{250} level. NUE generally had decreasing trend in response to increasing amounts of fertilizer-N in both investigated periods (Figure 5.1.6). The same trend was reported by Guarda et al. (2004), Bavec (1999) and Vukovic et al. (2008).

Nitrogen treatments and great within-treatment variability, probably due to variable residual soil N content, promoted a wide range of responses in plant growth and pigment content (Serrano et al., 2000). Significant differences in mean values of leaf TN content, CCI, TN content in grain, winter wheat yield, NUE and spectral indicators NDVI and RVI were obtained for two winter wheat cultivars/years, two growth stages under nine fertilization treatments (Table 5.1.2; Table 5.1.3). All investigated agronomic and spectral variables were strongly dependent on N application levels (Table 5.1.5; Table 5.1.6; Table 5.1.7) and increased with increasing N rates (ANOVA, $p < 0.001$), with consistent patterns across different cultivars and growth stages. The same significant dependency was reported by Barraclough et al. (2010) and Lopez-Bellido and Lopez-Bellido (2001). According to ANOVA for both cultivars/years and growth stages, it can be seen that three to four groups can be formed which means are significantly different from each other (Table 5.1.2). The reason for that can be natural variability that exists in the data set. Assuming that for significantly different fertilizer treatments this is an indication of the induced nitrogen stress, spectral response was later used in order to predict nitrogen treatment. Similar methodology was used by Alchanatis and Schmilovitch (2005).

When consider overall effect of different N fertilizer treatments, two winter wheat cultivars/years and two growth stages on leaf TN content, CCI, grain TN content, yield and NUE, mean differences between groups of N levels, cultivars and growth stages were statistically significant for every crop variable except NUE between cultivars/years (Table 5.1.4). Climatic and cultivar differences expressed as factor cultivar/year (one cultivar per one vegetation year) were statistically significant for leaf and grain TN content, CCI and yield. For example, when analyzing overall effect of N fertilization factor on mean differences of leaf TN content, four statistically different groups can be distinguish: 1) Control, N_0; 2) N_{100}; 3) N_{150}, N_{200}; 4) three combinations of N_{250}; N_{300} kg ha^{-1}. Results of ANOVA show significant treatment x growth stage (GS) interaction registered only for CCI for vegetation period with cultivar “Lucija”, which indicated that CCI responses to N application during 2010 varied across the two growth stages (Table 5.1.5). Growing conditions and crop genetic properties significantly affected harvest variables, leaf TN content and CCI for each growth stage (Table 5.1.7). A significant treatment x cultivar/year interaction was found for yield, grain TN content and leaf TN content at heading stage, which means that climate conditions and cultivars had different responses of measured variables under various N fertilization treatments.

Significant interactions cultivar/year x GS and treatment x cultivar/year were obtained only for leaf TN content which indicated that growing conditions depended on climate and soil-water regime, and that N fertilization level had significant influence on cultivar performance (Table 5.1.8). The absence of treatment x cultivar/year x GS interaction indicated that leaf TN and CCI responses to different N levels observed at two growth stages were similar for two growing periods/cultivars, even though significant variations in winter wheat variables exists between years. As seen in Table 5.1.6, cultivar "Lucija" achieved specific spectral responses in form of vegetation indices RVI and NDVI at stem extension stage to different N fertilization rates. The effect of N treatments on VIs was statistically significant ($p < 0.001$).

The hyperspectral data obtained by field spectroscopy reflected the complex information of crop growth, so several techniques and methods have been used to minimize spectra noise, such as derivative spectra and vegetation indices based on high correlation coefficients between spectra and winter wheat variables.

Visual evaluation of reflectance spectra and its first derivative indicates similar gross patterns of reflectance typical for vegetation (Figure 5.2.1a) (Zhu et al., 2008). Chosen spectra that best discriminated between treatments were acquired at stem extension stage from flag leaf of „Lucija“ cultivar. However, differences between three fertilization categories are obvious. Reflectance was increased at near-infrared region (NIR) (> 740 nm) and decreased at red region (660 – 690 nm) for group N_{250} and N_{300}. Nguyen and Lee (2006) reported the same spectral changes in rice leaf tissue. The reason for that is higher water content in leaves and increased CCI and leaf TN content which was also reported by Hansen and Schjoerring (2003). Group with N_{100}, N_{150} and N_{200} showed similar pattern in green and red part of spectrum, but had lower reflectance in NIR region. Nitrogen limitation in group with Control and N_0 highly increased red reflectance and decreased NIR reflectance, which was also found by Serrano et al. (2000) who estimated winter wheat yield under different N supplies. The greatest spectral differences between treatments were in the visible and at the red edge region. That was emphasized by changes in the position and amplitude of the first derivative of the reflectance (Figure 5.2.1b). Leaves without N fertilization showed a red edge shift to a shorter wavelength. The same pattern was found by Penuelas et al. (1994) and Zhao et al. (2005). This has the effect of broadening the green reflectance peak (normally located near 550 nm) towards longer wavelengths, increasing visible reflectance (Adams et al., 1999), and causing the tissues to appear chlorotic. Under low chlorophyll and TN content in the leaf (e.g. due to drought or crop senescence) sensitivity is greater in absorption peaks (450, 670 nm). In contrast, for leaves with greater CCI/leaf TN content, these absorption peaks become saturated, and the most sensitive spectral bands are placed around 550 nm (Penuelas et al., 1994). Changes in spectral signatures were captured between growth stages as well (see APPENDIX: Figure 9.2). Stem extension (F8) showed higher mean reflectance in the NIR region and decreased mean reflectance in the red region compared to heading (F10.5). Explanation for this pattern lies in lower chlorophyll content in leaves during latter growth stage. Absorbance of winter wheat flag leaves for both measurements during 2010 with cultivar „Lucija“ showed differences among N fertilization treatments and confirm earlier defined spectral regions sensitive on N supply (see APPENDIX: Figure 9.3; Figure 9.5). Absorbance measured at heading stage clearly distinguished among different N treatments according to the range of values for agronomic variables obtained according to the treatments (highest values at treatment VII) (Table 5.1.2). During stem extension stage, treatments without N strongly differ from the rest, probably due to fast plant development which required N supply and thus respond with higher chlorophyll content in leaves. Figure 9.4 (see APPENDIX) showed that differences between growth stages were largest at higher N fertilization rates. Comparison of treatment IX (N_{300}) among cultivars/years indicates better discrimination of GS in 2008, probably due to unfavorable climatic conditions in term of drought during stem extension which was reflected in further weaker plant development and maturation. Following the goals of this study, it can be concluded that the overall level of spectral reflectance was directly influenced by different N fertilization treatments as proved by Hansen and Schjoerring (2003). Increased N supply and growth stage F8 caused increased greenness, thus, reflecting high values of reflectance factor in the NIR region and low values in the red part of spectrum.

Dynamic patterns of flag leaf reflectance under varied N fertilization levels with two winter wheat cultivars provided basis for the analysis of sensitive wavelengths and VIs and deriving quantitative relationships of crop variables to spectral features of reflectance.

Data pooled from measurements acquired at stem extension during 2010 with cultivar „Lucija“ were used to perform correlation analysis between agronomic variables and spectral features.

From Table 5.3.1, a very strong to full correlation between leaf TN content, CCI, grain TN content, yield, NDVI and RVI is evident. Particularly, correlations between leaf TN content and CCI (r = 0.922), and NDVI and yield (r = 0.905) can be emphasized. Significantly high correlation between Chl readings and N content in leaf dry matter was found during stem extension and heading stage in winter wheat (Filella et al., 1995; Fox et al., 1994; Evans, 1983). Blackmer et al. (1994) reported good correlations between leaf reflectance in the visible part of the spectrum using chlorophyll meter readings, with leaf N and grain yield. Therefore, no attempts were made to study separately each of the biochemical winter wheat variables. Yield was the selected variable for further regression and classification modeling.

Wavelength dependence of correlation coefficient (r) between winter wheat yield, leaf TN content, CCI, grain TN content and leaf spectral reflectance, 1st and 2nd derivative of reflectance acquired by the field spectroradiometer at stem extension during 2010 is shown in Figure 5.3.1 and Figure 9.6abc (see APPENDIX). Spectral features of raw reflectance at specific wavelengths in the ranges 764-800 nm showed high positive correlation, and in ranges 510-662 nm and 689-731 nm high negative correlation with winter wheat yield. Significantly high positive correlation with yield achieved wavelengths in the ranges 560-677 nm and 719-767 nm, while negative correlation was found in the ranges 488-554 nm, 683-710 nm and in NIR region from 785 up to 1000 nm, when analyzing first derivative of reflectance. Abdel-Rahman et al. (2010), Penuelas et al. (1994), Filella et al. (1995), Read et al. (2002) and Zhao et al. (2003) reported similar spectral regions significant for predicting N status in crops. Most of these wavelengths are in the visible and red edge part of spectrum indicating chlorophyll optical properties. Leaf optical properties in a relatively narrow spectral band in the far red are crucial for plant nitrogen stress detection and the estimation of leaf chlorophyll concentration (Lichtenthaler et al., 1996). Close relationship between spectral and agronomic variables in winter wheat was found when leaves were green (F8). The relationship was weaker during heading stage (F10.5) (data not shown). When discussing correlogram for single wavelength of raw reflectance, the maximum correlation coefficient for leaf TN content was located at 557 nm and 716 nm (r = -0.78), for CCI at 713 nm (r = -0.82), for grain TN content at 767 nm (r = 0.71) and for yield at 557 nm (r = -0.90). Correlation analysis for first derivative of reflectance showed maximum *r* for leaf TN content at 602 nm and 671 nm (r = 0.83), for CCI at 602 nm (r = 0.86), for grain TN content at 896 nm (r = -0.89) and for yield at 587 nm (r = 0.93). Wei et al. (2008) also found a very close relationship between leaf nitrogen accumulation in winter wheat and red edge position.

Leaf TN content, CCI, grain TN content and yield showed very similar patterns which can be explained by high correlation coefficients between these four variables. The first derivative transformation showed higher overall *r* values than the corresponding raw reflectance. The *r* values for correlations of investigated agronomic variables and the first derivative of reflectance can be found in the following order: CCI > leaf TN > yield > grain TN.

Furthermore, the highest correlation coefficients for winter wheat yield in the red to red edge and NIR region indicated spectral bands for calculation of common VIs (NDVI and RVI) (algorithms shown in Table 4.5.3.1.1). According to the Jordan (1969) and Rouse et al. (1974) who used red and NIR bands for VIs combination and gave basis for spectral ratios application in estimating plant variables, NDVI and RVI were calculated from 704 nm (λ_{RED}) and 785 nm (λ_{NIR}). These two wavelengths exhibited the highest single wavelength correlation coefficient in each of the distinct high correlation spectral regions (red to NIR). The ratio and difference based VIs with similar spectral features were found by Carter (1994), Barnes et al. (2000), Moges et al. (2005) and Stone et al. (1996) who reported high correlations with crop chlorophyll content, N status and grain yield. Tarpley et al. (2000) found that ratios between red-edge (700 or 716 nm) and near-infrared (755–900 and 1000 nm) provided the best correlation with leaf N concentrations in cotton.

Osbourne et al. (2002) concluded that NIR region was crucial for estimation of grain yield. Using these results, a simple linear regression model was derived for wheat yield estimate and forecast based on NDVI integration during the wheat stem extension stage. The same statistical approach was used by Benedetti and Rossini (1993). Figures 5.3.2 and 5.3.3 show very strong positive and significant relationship between narrow band spectral indices and winter wheat yield (F8-2010-"Lucija") ($p < 0.05$). Correlation between grain yield and NDVI and RVI achieved $r = 0.91$ and $r = 0.87$, respectively. It can be seen that treatments with no N added (Control and N_0) are scattered in separate cluster (lower values around fitted line). Obtained results agree with Girma et al. (2006) who found that both the correlation and regression analysis suggested mid-season (F5, F7 and F10) NDVI, among Chl content and total N uptake, as good predictor of winter wheat grain yield. NDVI and RVI values increased with N fertilizer level as found by Lukina et al. (2000) (data not shown). A high correlation ($r = 0.75$) was observed between NDVI and grain yield by Goel et al. (2003) as well. Significant but medium positive correlations were found between the same NDVI and RVI and winter wheat yield ($r = 0.47$ and $r = 0.45$, respectively) at heading growth stage (F10.5–2010-"Lucija") ($p < 0.05$) (Figure 5.3.4; Figure 5.3.5). Lower correlations between VIs and grain yield in this growth stage could be result of N translocation to grain as well as influence of more intensive green color and higher leaf water content in stem extension. The obvious growth stage dependence of calculated VIs could be an advantage for precision farming applications.

The next objective of this study was to compare the predictive ability of different statistical methods and algorithms used to estimate winter wheat variables. Parametric empirical models – SLR, MLR and PLSR – and non-parametric computational models - ANN – were calibrated, tested and compared to predict yield variable from flag leaf spectral data in the form of first derivative of reflectance captured at stem extension stage from cultivar „Lucija". MLR models were tested for all measurements, all forms of spectral data and all crop variables. First derivative of reflectance resulted in an improved relationship between leaf reflectance and winter wheat agronomic variables than did raw reflectance and second derivative spectra. Zhao et al. (2005) recognized first derivative of spectra as best transformation which revealed wavelength features significant for prediction of winter wheat grain protein content. Whereas yield MLR model showed the best performance, this variable was selected for further analysis as input in above mentioned models. The main concept of modeling process is shown on Figure 5.4.1. Patterns of the spectral and biophysical data were modeled and calibrated to be applied to future data in order to predict the condition of winter wheat. PCA of the first derivative of reflectance resulted in 16 uncorrelated significant principal components (PCs). Factor scores acquired from the calculated PCs were used as predictor variables in a MLR and ANN modeling. 97.92 % of sum of squares has been explained by all the extracted components. First principal component (PC1) captured 62.98 % of the variability in the spectral data and 12.33 % of the variance was explained by PC2. However, this trend decreased with more components added to the model (see APPENDIX: Figure 9.7). The PCs provided statistical measures whereas VIs required *a priori* knowledge of the relations between spectra and the specific biophysical variables.

Detailed results of MLR model quantifying relationship between spectra and winter wheat yield represented on Figure 5.4.1.1 show very strong dependence among variables. Based on the 7 PCs which account for the most of the variation in spectral data, validation model (scatterplot upper right) which yielded a minimum root mean square of prediction (RMSEP) and the highest coefficient of determination (R^2) was built. The root mean square of calibration (RMSEC) was 3.60 dt ha^{-1}, while the linear regression coefficient R^2 between the measured and predicted grain yield was 0.89. The respective R^2 for the full cross validation was R^2 = 0.83 with RMSEP = 4.56 dt ha^{-1}. Model adequately predicted variations in grain yield, so noted residual variations should be due to noise only. Few samples with larger residuals than the others did not disturb the model to a large extent. It can be concluded that residuals were generally randomly distributed. Line plot of regression coefficients checked the importance of the different spectral components in predicting yield (PC1, PC2 and PC7). MLR models based on 5 PCs were performed for leaf TN content, CCI and grain TN content as well using the same data acquired at the same date (F8 – cultivar „Lucija“) to show which variable model had the highest accuracy performance. The reported results are similar (Figure 5.4.1.2). Among all four winter wheat variables, grain TN content estimation model had the highest coefficient of determination

(R^2 = 0.85), followed by yield (R^2 = 0.83), CCI (R^2 = 0.72) and leaf TN content (R^2 = 0.69), indicating that harvest variables had the best predictive power from spectral measurements at stem extension stage. The RMSEP were 0.383 %, 6.392 and 0.08 % for leaf TN content, CCI and grain TN content, respectively. The results of the models evaluation showed that using hyperspectral data, particularly first derivative of reflectance produced very high accuracy. Estimation models for the same winter wheat variables measured at the heading stage (F10.5) of cultivar „Lucija“ (2010) were less accurate compared to predictions modeled using spectra and variables acquired at F8 stage (see APPENDIX: Figure 9.10). Obtained results proved that the level of greenness captured by the spectral response during the largest wheat development was the best predictor of the potential harvest variables, which was also concluded by Wright et al. (2004). During the heading stage, CCI estimation models were the poorest according to the beginning of the N translocation from leaf to grain, which limited the ability of leaf reflectance to properly track changes in productivity. The R^2 values for full cross validated models were 0.67, 0.61, 0.56 and 0.54 for leaf TN content, grain yield, grain TN content and CCI, respectively. In early or middle growth stages, spectral modeling is useful for determining adequate nitrogen rate to achieve desired crop yield. In advanced and late growth stages, crop leaf/canopy spectral reflectance measurements can be used to monitor crop health and forecasting yield (Strachan et al., 2002). Compared to the measurements acquired from cultivar „Lucija“ during 2010, model performance for estimating the same four winter wheat variables from cultivar „Fiesta“ during 2008 was less accurate (see APPENDIX: Figure 9.8; Figure 9.9). The reason for that can be found in weaker crop development in 2008 due to the damages caused by water deficit in soil during stem extension and wildlife destructions which altogether resulted in reduced yield and earlier senescence, especially on N-stressed treatments. Correlations between predicted and measured values of leaf TN content, CCI, grain TN content and yield for validation model were strong to very strong (R^2 from 0.53 to 0.65) and medium to strong (R^2 from 0.17 to 0.38) for stem extension (F8) and heading (F10.5) stage, respectively.

Very strong relationship was found between observed and predicted NUE ($r = 0.81$; $p < 0.05$) (Figure 5.4.1.3). NUE, as agronomic indicator was calculated using measured data of grain TN content and yield of cultivar „Lucija“. The algorithm was reported in subheading 4.5.3.2. Predicted NUE was calculated based on the PLSR full cross validation results for harvest data, and then regressed against the observed values. This relationship is the most encouraging result due to the high predictive ability of the models based on spectral data to accurately estimate winter wheat harvest variables.

The PLSR results showed that it is possible to predict grain yield using hyperspectral field spectroscopy data. Jensen et al. (2007) found very high correlations between predicted and measured values for the calibrated and validated samples (r = 0.97 for winter wheat yield and grain protein models). Full correlation between predicted and measured values for the calibrated and validated samples was r = 0.93 and r = 0.91, respectively (Figure 5.4.1.4). An 8 factor PLSR model was found to yield a RMSEC of 4.10 dt ha^{-1}. Validation of the model with in-field acquired data showed that winter wheat yield can be predicted with a RMSEP of 4.61 dt ha^{-1}. Score plot showed indication of clustering in the set of spectral samples which means that samples within each of three clusters are similar. Spectral features projected right from the center are positively linked to winter wheat yield (green and red edge region). Wavelengths projected in the opposite direction had a negative link (blue and the NIR region).

Wavelengths with large loading weight values were important for the prediction of the winter wheat yield (Figure 5.4.1.5). Visible part of spectrum from approximately 550 nm to 670 nm and 690 nm to 710 nm with red edge region from 730 nm to 770 nm were identified as zones of major importance for the PLSR model of winter wheat yield. Visible wavelengths were directly associated with chlorophyll absorption. High degree of coincidence was found between the selected wavelengths for the best NDVI and RVI index, correlations between each single wavelength and yield, and the size of the numerical PLSR regression coefficients. The similar conclusions were obtained by Hansen and Schjoerring (2003). Model adequately predicted variations in grain yield, so residual variations shown in Figure 5.4.1.5 should be due to noise only. Residuals were generally randomly distributed although they formed two „clouds“ due to differences in winter wheat yield values. Small formation of residuals (left) represent samples with no N applied (Control and N_0 treatments) which achieved reduced yields.

Despite being an empirical approach, the model obtained by PLSR calibration was able to integrate physiological information from number of spectral bands in order to estimate winter wheat yield (Ferrio et al., 2005). Using PC explanatory analysis and PLSR, Moron et al. (2007), among others, agreed that VNIR reflectance spectroscopy is a suitable method to assess nitrogen status in fresh winter wheat samples.

The next statistical method to quantitatively estimate grain yield from spectral data was back propagation neural network model, a kind of artificial neuron network (ANN) as non-linear technique. Predicted vs. observed winter wheat yield relationship for train (calibration) and test (validation) dataset explained using ANN regression modeling was found to have full correlation: r = 0.95 and r = 0.92, respectively (Figure 5.4.2.1, Table 5.4.2.1), which indicated a good learning performance. Model performance was assessed by SOS error function which showed how closed the ANN predictions were to the measured yield. Training and testing data sets were split between themselves in ratio 50:50 which resulted in a good random distribution of wide range of yield values, and achieved excellent target estimation. Predictions of winter wheat yield in train and test samples had the largest residuals for treatments III-1, VI-4 and I-3 and III-2, respectively (Table 9.4 and 9.5). This could be simply explained by natural and expected variations in sampled yield per each treatment, and partly due to the model error.

The most interesting review of obtained results is analysis of the overall success of winter wheat yield prediction, assessed by different models (Table 5.4.2.2). Results showed that optimal ANN model gave the highest coefficients of determination (R^2) and the lowest root mean squared errors (RMSE) than the corresponding SLR-VIs, MLR and PLSR models, in both calibration and validation tests. Chen et al. (2007) obtained similar results of comparison between regression models and ANN when estimating rice pigment content from hyperspectral data. The highest RMSE of prediction was found for simple linear regression model of RVI and yield (RMSEC = 5.44 dt ha^{-1} and RMSEP = 5.75 dt ha^{-1}). Accuracy was reduced for cca. 23 % (validation set) compared to the ANN estimation. The next best performance was achieved by MLR model. The corresponding RMSE for the both calibration and validation was 3.60 dt ha^{-1} and 4.57 dt ha^{-1}. Differences between MLR and PLSR regression parameters were negligible according to the purpose of yield prediction and in-season N rate optimization. Performance of the PLSR model indicated the highest consistency because of the small difference between RMSEC (4.10 dt ha^{-1}) and RMSEP (4.61 dt ha^{-1}) besides high prediction ability (validation $R^2 = 0.84$). PLSR makes full use of the rich hyperspectral information while being relatively insensitive to sensor noise (Atzberger et al., 2010). The PLSR model performed better (up to 20 %) compared to the best of the selected VIs based on linear curve fitting, showing that PLSR is a potentially useful explorative tool which was also proved in results reported by Hansen and Schjoerring (2003). The values obtained for calibration and validation by both models are comparable which indicates that both models were able to learn and generalize. Comparing MLR, PLSR and ANN, the latter model outperformed the first two. This might be resulted from the strong capacity for nonlinear mapping and good robustness of ANN, which maximized the sensitivity to the yield data of the first derivative of reflectance. Owing to the simple topological structure, the learning algorithm corresponds to the solution of a linear problem, and therefore, the training of the network is less time-consuming (Yang et al., 2009). The ability of ANN to associate complicated spectral information with target attributes without any constraints for sample distribution make them ideal for describing the intricate and complex non-linear relationships which exist between canopy-level spectral signatures and various crop conditions (Kimes et al., 1998). Given the complexity of estimating crop yield and harvest variables based on spectral data, the above RMSEP values can be considered very small as stated by Uno et al. (2005). Considering coefficients of determination, performance of two vegetation index-based models could not reach the accuracy of the multivariate regression models, despite latter required empirical calibration. The same relation in model performance was proved by Ferrio et al. (2005) and Atzberger et al. (2010). Still, NDVI and RVI reached a very strong relationship with yield when look on the cross-validation results: $R^2 = 0.80$ and $R^2 = 0.73$, respectively. Ordinary kriging results shown on Figure 5.4.2.2 indicate similarities between the maps generated from the equations and the one generated from field measurements, which is in agreement with high portion of yield variability explained by spectral data ($p < 0.05$). Disagreements mostly occurred on treatments VI, VII and VIII due to the high variability in the yield data within the same treatments. Maps generated from all models except PLSR had smaller areas of high yield. Yield was over-estimated by MLR, PLSR and in small portion by ANN model. NDVI and RVI estimates were considerably lower than the measured grain yield and had the highest disagreements in spatial distribution with measured values according to higher prediction errors (Table 5.4.2.2). ANN map was the closest to measured winter wheat yield considering spatially comparable estimates.

Under the diverse experimental conditions including different N fertilization levels and combinations, it can be concluded that the methods used in this study can be used to provide in-season estimates of winter wheat yield at a field scale using remotely-sensed observations. Results showed that VIs and both statistical and ANN methods could be used to estimate the in-field variation of yield potential. Moreover, MLR showed very high correlations with the rest of agronomic and biochemical winter wheat variables (leaf and grain TN content, CCI).

Classifying crops by nitrogen stress is of great interest in precision agriculture. Following that assumption, classification analysis based on spectral features of fresh winter wheat flag leaves were computed as well. Discriminant function analysis (DA), clustering and ANN classification task were initiated and processed to find N level membership. Standard statistics reported in the Table 5.5.1.1 was used to denote the statistical significance of the discriminatory power of the current model and unique contribution of the respective spectral PC to the discrimination between three groups of N fertilization levels. Nine PCs were marked to have significant contribution to N levels discrimination ($p < 0.05$). Two statistically significant discriminant functions (roots) were selected after computing canonical analysis (Table 5.5.1.2). The first function accounted for over 99 % of the explained variance which means that 99 % of all discriminatory power was explained by this function. Apparently, the first discriminant function discriminated mostly between the group I (Control, N_0 kg ha^{-1}) and the other N treatments categorized in groups II and III (Table 9.1). The second discriminant function distinguished mostly between the group II (N_{100}, N_{150}, N_{200} kg ha^{-1}) and the other N treatments in groups I and III. However, based on the review of the eigenvalues in the Table 5.5.1.2, the magnitude of the discrimination was much smaller. The plot on Figure 5.5.1.1 confirmed the interpretation so far. The first discriminant function mostly discriminated between group I and the other two, while second function showed discrimination between the group II and the others. Table 9.2 shows prediction accuracy of N treatment group classification using DA. All samples were correctly classified in each group by the current classification functions. However, the interpretation of these results in terms of predictive discriminatory power is possible only with validation of the discriminant function analysis results with new additional data. In general, in many cases where there are naturally occurring groups that could be discriminated, this technique is appropriate. These results indicated that hyperspectral measurements are best at detecting where N is limited rather than where it is in excess during the fast vegetation development (F8), which was also reported by Jensen et al. (2007) who estimated wheat harvest variables under different N fertilization levels. The similar results were reported by Strachan et al. (2002) who concluded that these findings are of great interest in precision agriculture by allowing supplemental nutrient application, identifying stress patterns and aid in yield forecasting. Early in the growing season, N could still be applied in accordance with plant's needs. Under such a precision agriculture – variable rate application strategy, remotely sensed information would be combined with other characteristics of the field (topography, drainage patterns, soil pH etc.) in the management decision to determine the potential economic benefit to any N application. Karimi et al. (2005) also used discriminant analysis of hyperspectral data for assessing water and nitrogen stresses in corn and reported that treatments were correctly classified with more than 95 % accuracy based on specific narrow wavebands from different portions of the spectrum.

The next classification methods used for detecting N status of winter wheat was clustering. ANOVA showed that nine fertilization treatments could form three groups in which the means of winter wheat variables (cultivar "Lucija", 2010) are significantly different (Table 5.1.2). According to that separation of N levels, leaf spectral response (PCs of the first derivative of reflectance) acquired at stem extension 2010 was used in order to cluster the data by fertilizer treatment group. Results (Figure 5.5.2.1) showed almost completely discrimination between treatment groups 1 (N_{250}, N_{300} kg ha^{-1}), 2 (N_{100}, N_{150}, N_{200} kg ha^{-1}) and 3 (Control, N_0 kg ha^{-1}), similarly to the results of the discrimination analysis. Part of the observed variability is due to the natural variability that exists in the data set (different levels of winter wheat variables in the same N treatment) and part due to the model error (Alchanatis and Schmilovitch, 2005). Two intermediate treatments (V-2, V-4 - group 2) got into group 1, and one sample from group 1 (IV-4) was close to group 2. The reason for that is a wide range of N-non-limited yield values. Loadings location in the PC space corresponds to cluster pattern on the score plot. For example, N limited treatments (group 3) can be distinguished from other groups mostly based on the blue, green and the red edge region due to the changes in chlorophyll content detected in these particular regions. Sample I-1 seemed to be an outlier. However, all samples were spread in one wide "cloud" and that particular variable is not so far from the center. Analyzing overall success of cluster analysis, it can be concluded that statistically different groups of N treatments were separable based on the spectral data. Multivariate discriminant analysis and clustering based on the spectral PCs clearly separated winter wheat leaves of different N status. Discriminant scores could be used to predict physiological conditions from leaf hyperspectral measurements, and similar approach could be tested at canopy or large-scale levels (Penuelas et al., 1994).

As a last statistical analysis, ANN was trained and tested to solve classification problem of discriminating different N fertilizer levels based on the leaf hyperspectral reflectance. By contrast to regression problems, a neural network classifier in this case assigned class membership to a three above selected input categories: I (Control, N_0), II (N_{100}, N_{150}, N_{200}) and III (N_{250}, N_{300}) (kg ha^{-1}). ANN type was MLP, also used for regression task. Activation functions were identity and softmax, usually used for classification analysis (Table 5.5.3.1). Prediction accuracy of ANN is reported in Table 5.5.3.2 and Table 9.3. Only one variable was misclassified in group II representing group III (V-1, test set) indicating 91 % statistically correct classified variables in the intermediate treatment group II. Correct classification of samples III-2, IV-2, V-2, and V-3 in the test data sets was not statistically significant (Table 9.3). As mentioned above, intermediate treatments (group II) had the highest variability in both laboratory measured crop variables and spectral data. As both DA and cluster analysis, ANN accurately classified different levels of N fertilization treatments in form of three statistically different groups. However, type of classification performed in this study was more explanatory and kind of pre-analysis. DA would need validation of obtained functions with a new data. ANN needs much more samples in the model to avoid over-fitting. Clustering gives review on spectral data pattern like PCA. But, this similar and enough accurate performance indicates potential of hyperspectral sensing to detect N limited and N excessed winter wheat conditions. Extreme groups were completely separable (I and III), and the small proportion of variability in intermediate N non-limited treatments (II) was probably result of some additional factors during the growing season like water stress and low soil pH.

Although the present study was carried out on two winter wheat cultivars of two growing seasons, the monitoring models for N status in crop tissue based on hyperspectral reflectance were established and tested in the same environment. Key hyperspectral parameters, regression and classification models still need to be verified in other ecological conditions involving different climate properties, soils, cultivars and production systems, and remain to be refined for accurate estimation and potential application in field management. Also, additional attempts should be made to integrate the air-borne and satellite remote sensing information and the present monitoring models from field spectroscopy data obtained in this study. This would help to extract spectral information related to monitoring and diagnosis of N status in wheat crop at larger scale, and further construct a potential platform for dynamic management of N fertilization (implementation of spectral model estimates into VRT applicator) and precise prediction of productivity for large spatial scale wheat production.

A comprehensive evaluation of thousands of simple ratio and difference based spectral indices constructed as all possible wavelength combinations would be required to determine consistent wavebands that provide the best information for developing more accurate monitoring models with wider applicability of winter wheat N status estimation. In further research extension in domain of hyperspectral data quantification, it is necessary to increase the amount, complexity and representation of samples so that the derived model can be applied under varied conditions. Accordingly, proper N monitoring models in practical use could be flexibly selected according to the specific growth stages of winter wheat.

As stated by Carver (2009) in the chapter "Nitrogen use efficiency as a driver of new technology", creating quantifiable methodologies to determine optimal rates of soil nutrient inputs that can be used both in intensive, large-scale, high-technology production systems and in developing world single-hectare farms is a daunting task. But, first, efforts must be taken to reach the producers by simplifying scientific-based decision making algorithms.

The results obtained in this doctoral thesis confirm the high information potential and feasibility of field spectroscopy for estimation of winter wheat conditions during development and harvest because they are scalable and applicable in high range of N stress and non-N-limited environments. Key spectral features and algorithms should help to support non-destructive and real-time monitoring of N status in wheat production by using hyperspectral remote sensing. Developed models represent basis for optimization of N use. This potential will only be realized when sensors and radiometric corrections would be optimized for agriculture (low cost, simplified algorithms, real-time and incorporated within VRT). Alternatively, the results of the spatial distribution of the in-season and harvest parameters could be used to change and improve management practices in the following crops (Jensen et al., 2007). However, it should be remembered that any nitrogen strategy should also take climatic conditions, as well as crop and soil history into consideration.

7 CONCLUSIONS

This doctoral study explored the potential of field hyperspectral measurements to develop in-season field-scale prediction of winter wheat variables and confirmed appointed hypothesis and recent scientific achievements.

1. Nitrogen treatments and great within-treatment variability promoted a wide range of responses in plant growth and biochemical constituents content. Considering all external effects, the expected influence of N fertilization on agronomic variables was recorded which was important for evaluation of spectral data and modeling. Significant differences in mean values of leaf TN content, CCI, TN content in grain, winter wheat yield, NUE and spectral indicators NDVI and RVI were obtained for two winter wheat cultivars/years, two growth stages under nine fertilization treatments. All investigated agronomic and spectral variables were strongly dependent on N application levels and increased with increasing N rates with consistent patterns between cultivars and growth stages. The absence of treatment x cultivar/year x GS interaction indicated that leaf TN and CCI responses to different N levels observed at two growth stages were similar for two growing periods/cultivars, even though significant variations in winter wheat variables exists between years. Cultivars responded similarly to N fertilization levels at both growth stages according to ANOVA calculated for overall effect. Results of ANOVA showed the absence of treatment x cultivar/year interaction for leaf TN content measured at F8 stage which indicates possibility for spectral sensing of winter wheat in stem extension stage regardless of cultivar differences.
2. The overall results on winter wheat variables indicated that varied N fertilization rates affected radiation reflection by crop leaf under different N treatments. The hyperspectral data obtained by field spectroscopy reflected the complex information of winter wheat growth. The treatment differences were enhanced by the different cultivars and growing conditions and created a wide range of variation in leaf N status and thus leaf spectral reflectance, so the quantitative relationships found should be applicable to diverse N nutrition levels. The greatest spectral differences between N fertilization treatments were found in the visible and the red edge region. Reflectance

from samples without N fertilization showed a red edge shift to a shorter wavelength. Due to the changes in chlorophyll content in leaves, leaf reflectance acquired at stem extension (F8) showed higher mean values in the NIR region and decreased mean values in the red region compared to heading (F10.5). The same pattern was recognized in the case of the higher N supply. The NIR and red reflectance contributed most to development of principal components which carried the most of variation in spectral data into the prediction analysis, and had the highest correlations with the winter wheat agronomic variables.

3. Correlation analysis between winter wheat agronomic variables and spectral data resulted in very strong to full relationships between leaf TN content, CCI, grain TN content, yield, NDVI and RVI [r (NDVI : yield) = 0.91]. High and robust correlations between spectral indices calculated from reflectance that was acquired at F8-“Lucija”, and all crop variables among each other for the same period were enough reason to choose yield variable as input to different prediction models development. Selection of stem extension stage for yield prediction gives opportunity for optimization of additional N topdressing for late maturing cultivars. Of course, further investigation should be focused on yield prediction based on spectral sensing at earlier winter wheat development stage like tillering.

   According to the single λ correlation analysis, close relationship between spectral and agronomic variables was found when leaves were in stage of increased vegetative growth (F8). Leaf TN content, CCI, grain TN content and yield showed very similar patterns which can be explained by high correlation coefficients between these four variables. The first derivative transformation showed higher overall *r* values than the corresponding raw reflectance. The highest correlation coefficients for winter wheat yield in the red to red edge and NIR region indicated spectral bands for calculation of common VIs. NDVI and RVI were calculated from 704 nm (λ_{RED}) and 785 nm (λ_{NIR}). Very strong positive and significant relationship was determined between narrow band spectral indices and winter wheat yield (F8-2010-“Lucija”) ($p < 0.05$). Correlation between grain yield and NDVI and RVI achieved r = 0.91 and r = 0.87, respectively. Growth stage dependence of calculated VIs indicates different wavelength selection.

4. Statistical (SLR, MLR, PLSR) and ANN approaches were adopted to develop winter wheat variables prediction models from flag leaf spectral data in the form of the 1st derivative of reflectance. The regression equations between spectral features and leaf TN content, CCI, grain TN content, yield and NUE were established. Factor scores acquired from the calculated PCs were used as predictor variables in a MLR and ANN modeling. First principal component (PC1) captured 62.98 % of the variability in the spectral data and 12.33 % of the variance was explained by PC2. Multiple linear regression identified a 7 PCs leaf reflectance model that explained 83 % of the variability in winter wheat yield, and accounted for a large variance in leaf and grain biochemical concentration ($R^2 \sim 0.8$, $P < 0.05$) (F8 – cultivar „Lucija“). Harvest variables had the best predictive power from spectral measurements at stem extension stage. Model evaluation showed that using hyperspectral data, particularly first derivative of reflectance produced very high accuracy. Results proved that the level of greenness and the higher leaf TN and Chl content captured by the spectral response during the largest wheat development is the best predictor of the potential harvest variables. During the heading stage, CCI estimation models were the poorest according to the beginning of the leaf senescence which limited the ability of leaf reflectance to properly track changes in productivity. The R^2 values of full cross validated models for variables acquired at heading stage were 0.67, 0.61, 0.56 and 0.54 for leaf TN content, grain yield, grain TN content and CCI, respectively. Weaker crop development during 2008 caused by water deficit in soil during stem extension and wildlife destructions resulted with less accurate predictive models of cultivar „Fiesta“ variables.
5. An 8 factor PLSR model results showed that it is possible to predict grain yield using hyperspectral field spectroscopy data. Full correlation between predicted and measured values for the calibrated and validated samples was $r = 0.93$ and $r = 0.91$, respectively. Visible part of spectrum from approximately 550 nm to 670 nm and 690 nm to 710 nm with red edge region from 730 nm to 770 nm were identified as zones of major importance for the PLSR model of winter wheat. The same regions were found after single wavelength and yield correlation analysis. Despite being an empirical approach, the model obtained by PC explanatory analysis and PLSR calibration was able to

integrate physiological information from number of spectral bands in order to estimate winter wheat yield. Results obtained for F8 stage during cultivar „Lucija“ development impose conclusion that forecasting the NUE by grain TN content and yield data estimated by field spectroscopy (using PLSR) is feasible (*r* for observed vs. predicted NUE was 0.81, $p < 0.05$).

ANN models were the most efficient in capturing the complex link between yield and leaf reflectance spectra (train and test dataset with r = 0.95 and r = 0.92, RMSEC = 2.57 dt ha^{-1} and RMSEP = 4.41 dt ha^{-1}, respectively) compared to corresponding SLR-VIs, MLR and PLSR models, indicating good learning performance. This might be result of the strong capacity for nonlinear mapping and good robustness of ANN, which maximized the sensitivity to the yield data of the first derivative of reflectance.

Performance of the PLSR model indicated the highest consistency because of the small difference between RMSEC (4.10 dt ha^{-1}) and RMSEP (4.61 dt ha^{-1}) besides high prediction ability (validation $R^2 = 0.84$). The PLSR model performed up to 20 % better compared to the best of the selected VIs based on linear curve fitting, showing that PLSR is a potentially useful explorative tool. Still, NDVI and RVI reached a very strong relationship with yield due to the cross-validation results: $R^2 = 0.80$ and $R^2 = 0.73$, respectively.

6. Classification analysis of N stress in winter wheat based on hyperspectral data (PCs) was performed using discriminant function analysis, clustering and ANN. According to the ANOVA results and mean comparison for variables measured in 2010 (“Lucija”), fertilization treatments were grouped into three statistically different categories of N rates which represented basis for classification analysis of spectral features. The first discriminant function mostly discriminated between group I (Control, N_0) and the other two (group II: N_{100}, N_{150}, N_{200}; group III: N_{250}, N_{250} + amendments, N_{300}) (kg N ha^{-1}). The second function showed discrimination between the group II and the others. All samples were correctly classified in each group by the current classification functions. Results indicated that hyperspectral measurements are best at detecting where N is limited rather than where it is in excess during the fast vegetation development (F8)

which is of great interest in precision agriculture by allowing N top-dressing, identifying stress patterns and aid in yield forecasting.

Cluster analysis found almost completely discrimination between the same treatment groups, similarly to the results of DA. Part of the observed variability was due to the natural variability that exists in the data set and part due to the model error. Spectra loadings showed that N limited treatments can be distinguished from other groups mostly based on the blue, green and the red edge region due to the changes in chlorophyll content.

Utilizing ANN classification analysis, 91 % variables in the intermediate treatment group II was correctly classified ($p < 0.05$), indicating the highest variability in both laboratory measured crop variables and spectral data in that specific category. As both DA and cluster analysis, ANN clearly separated winter wheat leaves of different N status in form of three statistically different groups.

Classification task performed in this study represent type of pre-analysis. Irrespective of this statement, enough accurate performance indicates potential of hyperspectral sensing to detect N limited and N excessed winter wheat conditions. Extreme groups were completely separable (I and III), and the small proportion of variability in intermediate N non-limited treatments (II) was probably result of some additional factors during the growing season.

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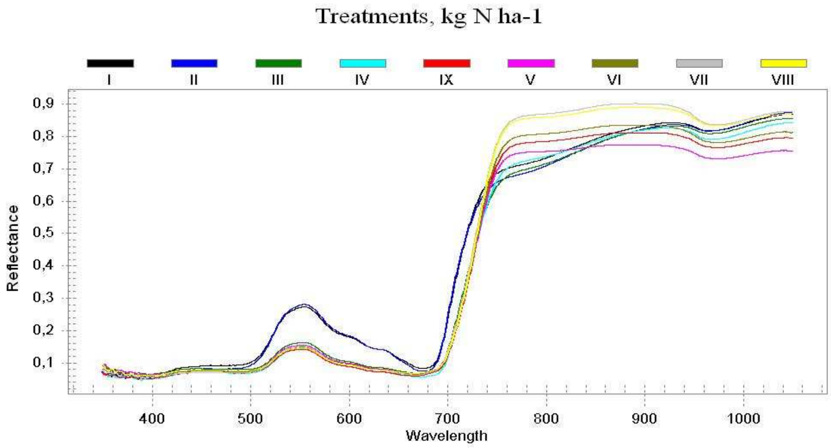
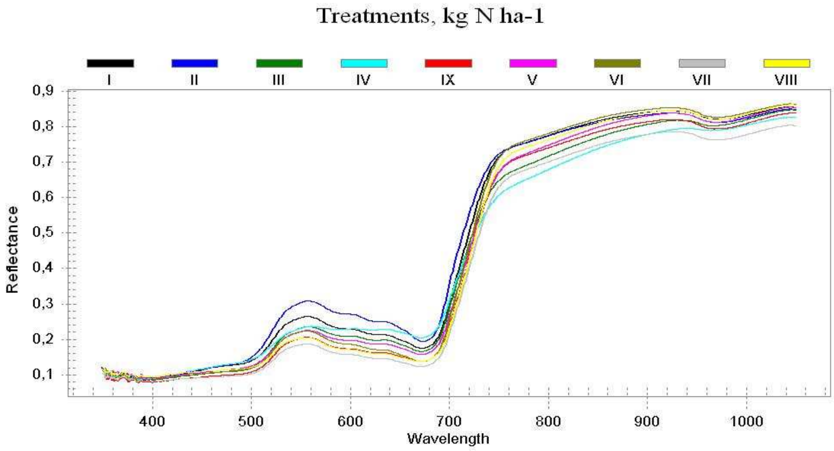
9 APPENDIX



F8-2010



F10.5-2010

Figure 9.1 – Average reflectance spectra of winter wheat flag leaves for different N fertilization levels acquired on May 07, 2010 (F8 - "Lucija") and June 05, 2010 (F10.5 - "Lucija") (n=36; reflectance expressed as reflectance factor).

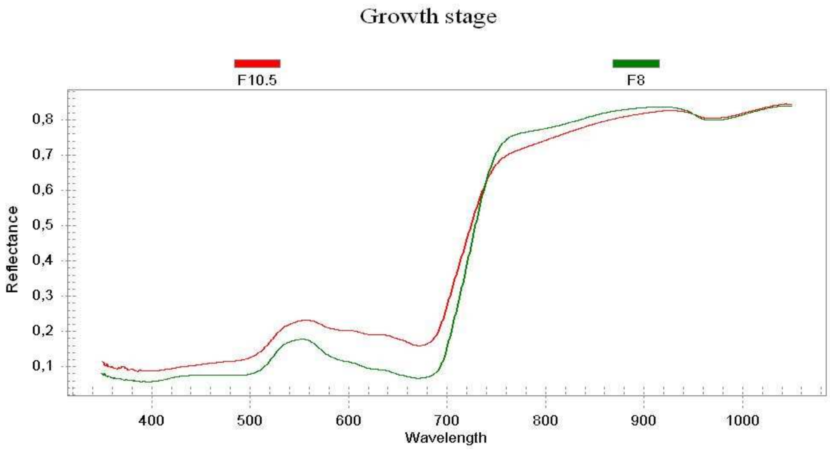
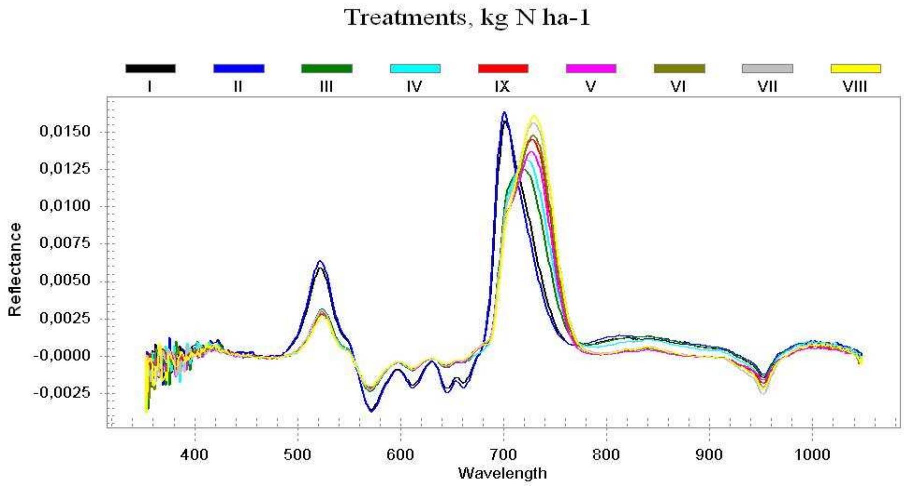
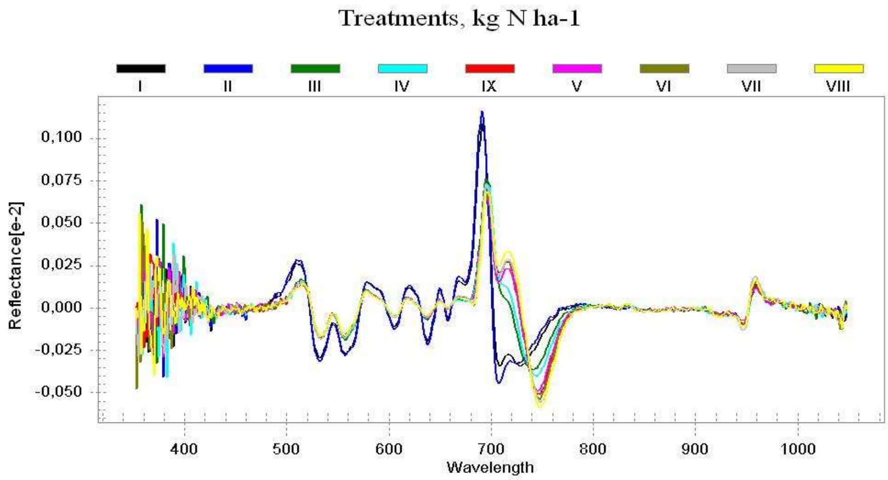


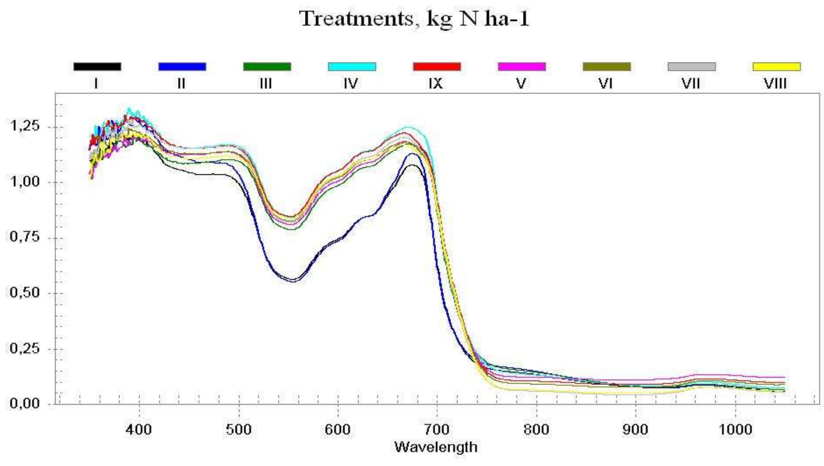
Figure 9.2 – Average reflectance spectra of winter wheat flag leaves representing two growth stages of cultivar "Lucija", acquired at May 07, 2010 (F8 – stem extension) and June 05, 2010 (F10.5 - heading) (n=36; reflectance expressed as reflectance factor).



F8-2010-dv1



F8-2010-dv2



F8-2010-abs

Figure 9.3 – Average of first and second derivative of leaf reflectance, and absorbance (log (1/R)) for different N fertilization levels acquired on May 07, 2010 (F8 - "Lucija") (n=36).

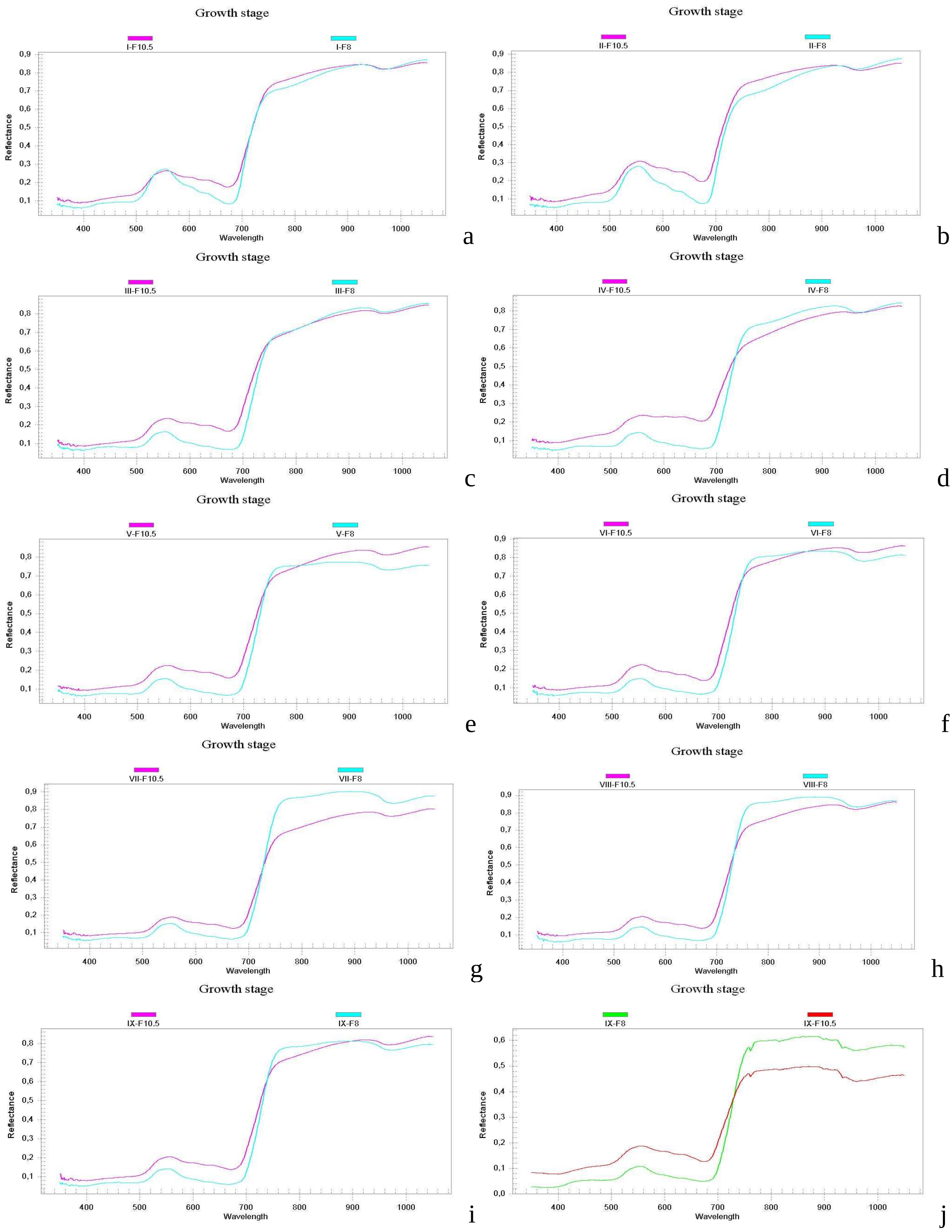
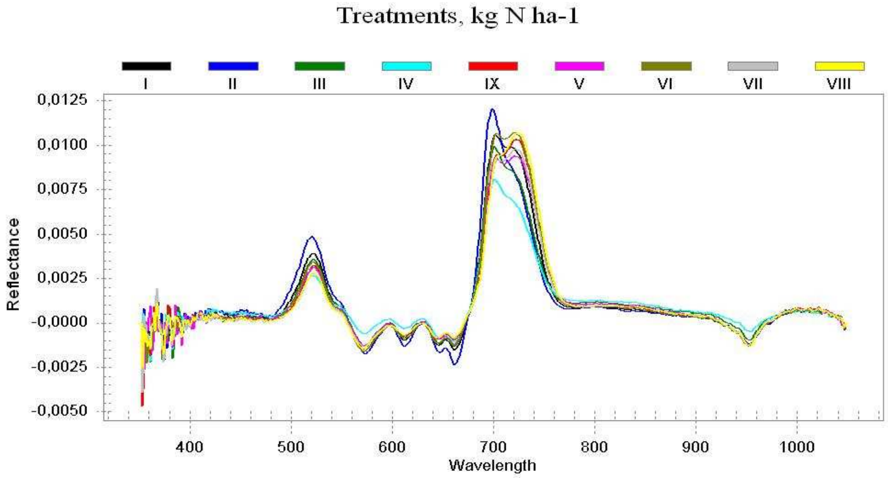
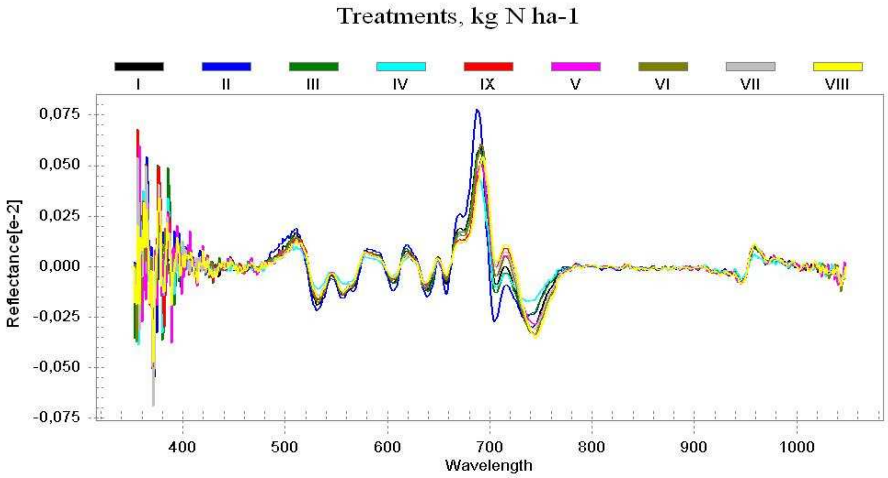


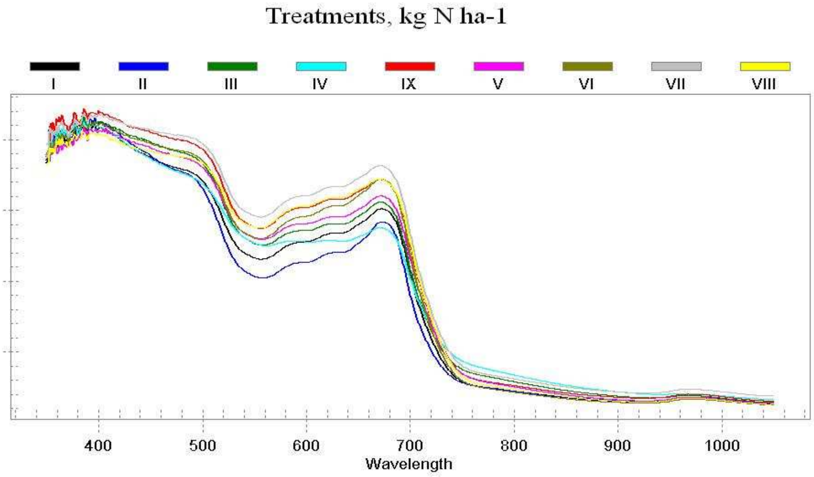
Figure 9.4 – Average leaf reflectance spectra representing two growth stages of cultivar "Lucija", acquired at May 07, 2010 (F8 – stem extension) and June 05, 2010 (F10.5 - heading) for each N treatment (a-i) (n=36; reflectance expressed as reflectance factor). (j) reflectance acquired at May 08, 2008 and June 10, 2008, treatment IX.



F10.5-2010-dv1

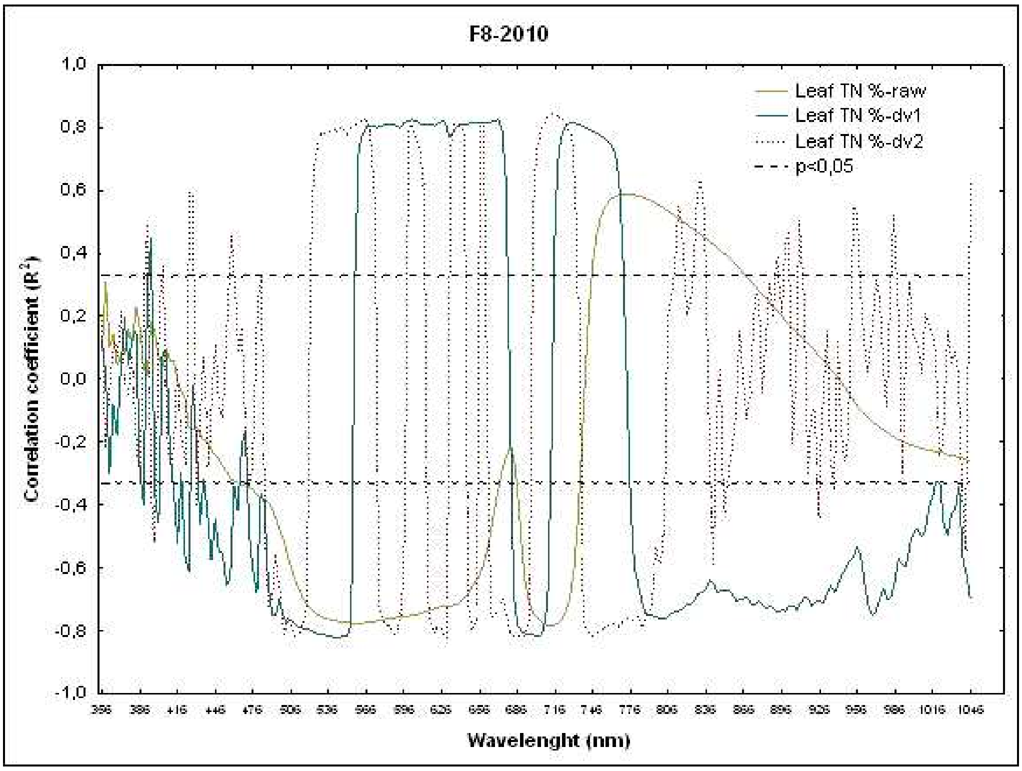


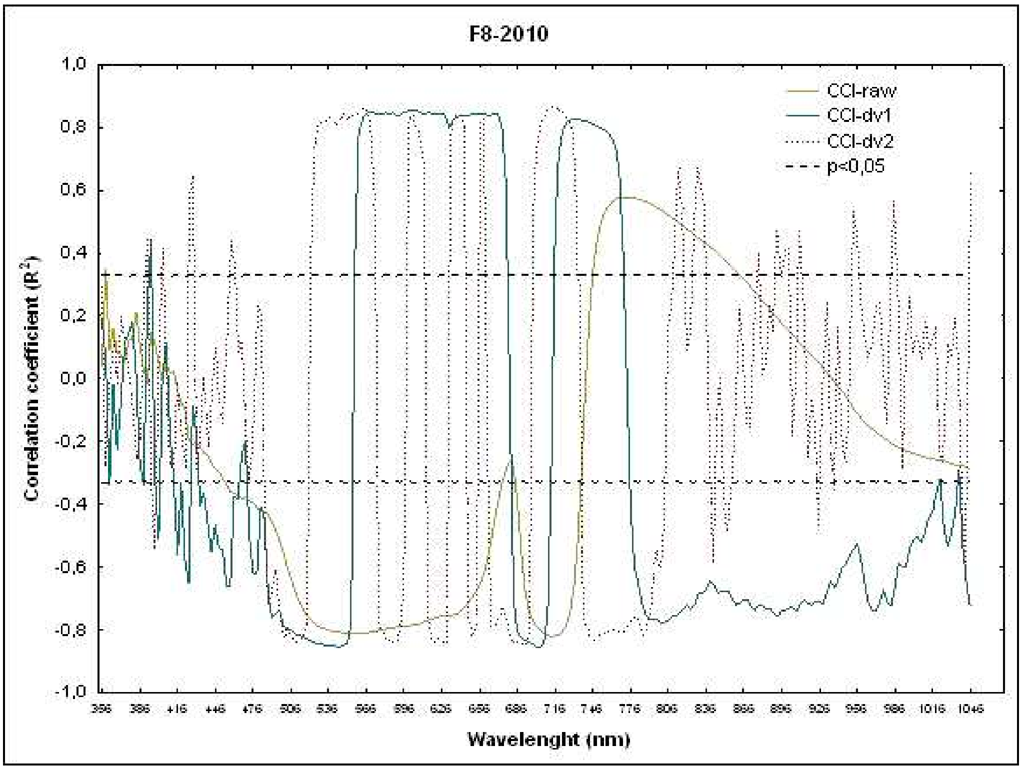
F10.5-2010-dv2

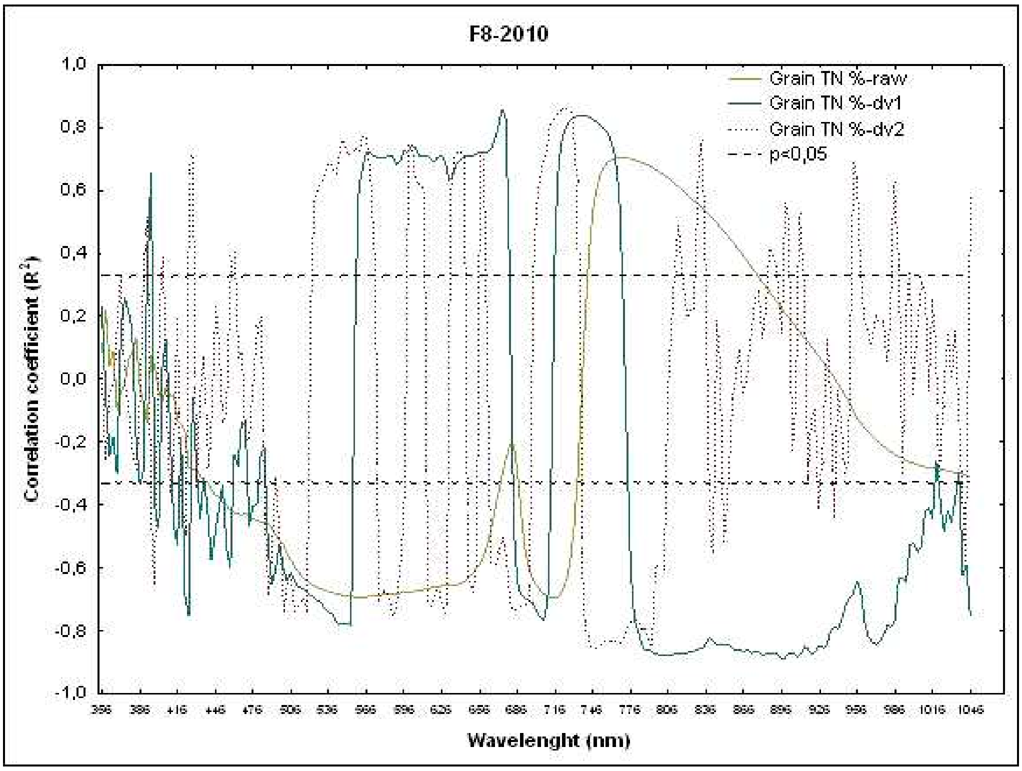


F10.5-2010-abs

Figure 9.5 – Average of first and second derivative of leaf reflectance, and absorbance (log (1/R)) for different N fertilization levels acquired on June 05, 2010 (F10.5 - "Lucija") (n=36).


F8-2010
Leaf TN %-raw
Leaf TN %-dv1
Leaf TN %-dv2
p<0,05
Correlation coefficient (R^2)
Wavelenght (nm)
a


F8-2010
CCI-raw
CCI-dv1
CCI-dv2
p<0,05
Correlation coefficient (R^2)
Wavelenght (nm)
b



c

Figure 9.6 – Single wavelength dependence of correlation coefficient (r) between winter wheat leaf TN content (a), CCI (b), grain TN content (c) and leaf spectral reflectance, 1st and 2nd derivative of reflectance acquired by the field spectroradiometer on May 07, 2010 (F8 – stem extension, "Lucija"/2010) (λ no.=231, range 356-1046 nm).

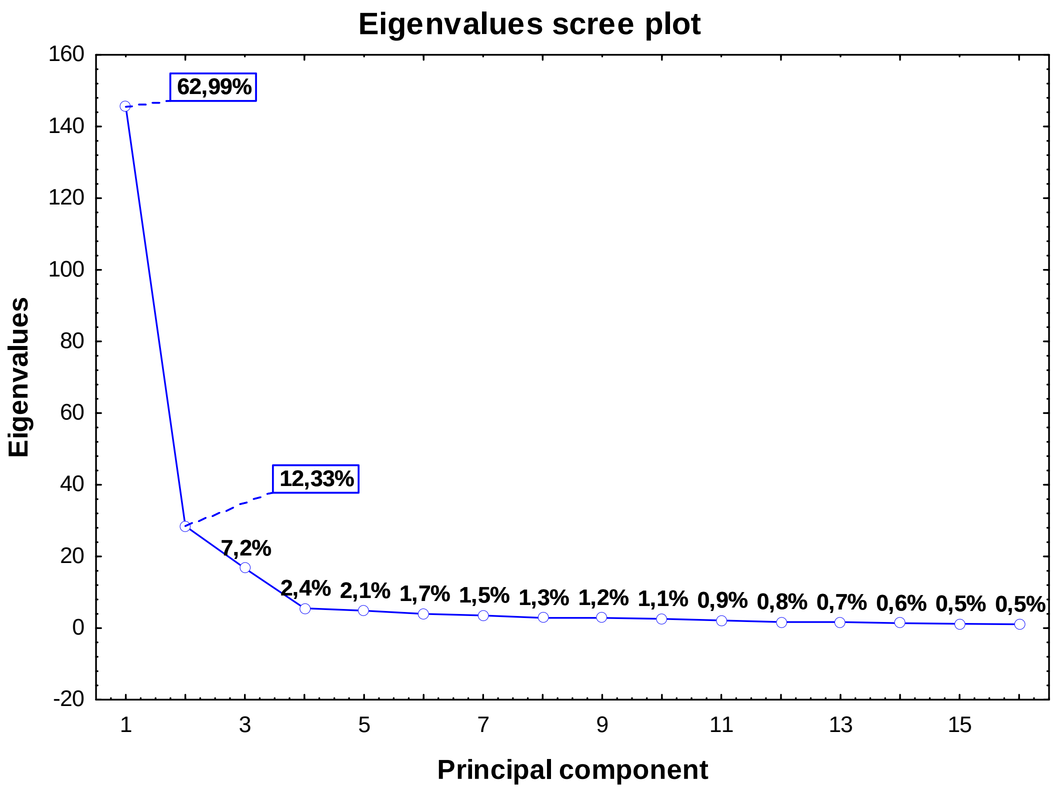


Figure 9.7 – Scree plot with eigenvalues showing total variance of spectral data in form of the 1st derivative of reflectance acquired at growth stage F8 - "Lucija"/2010 (λ = 231).

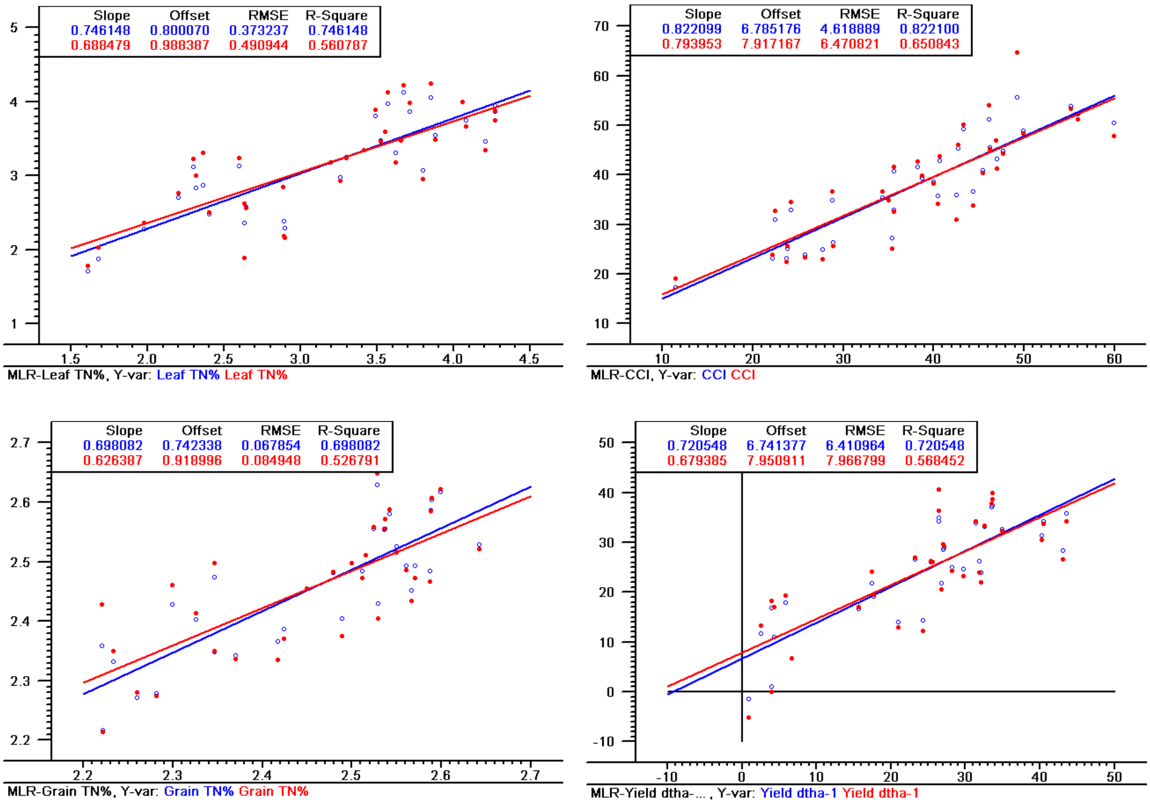


Figure 9.8 – Scatter plots of the MLR model performed using full cross validation method from 1st derivative of reflectance acquired at growth stage F8 - "Fiesta"/2008 showing relationship between predicted and observed winter wheat leaf TN content (%), CCI, grain TN content (%) and grain yield (dt ha^{-1}) (n=36). ● calibration ● validation

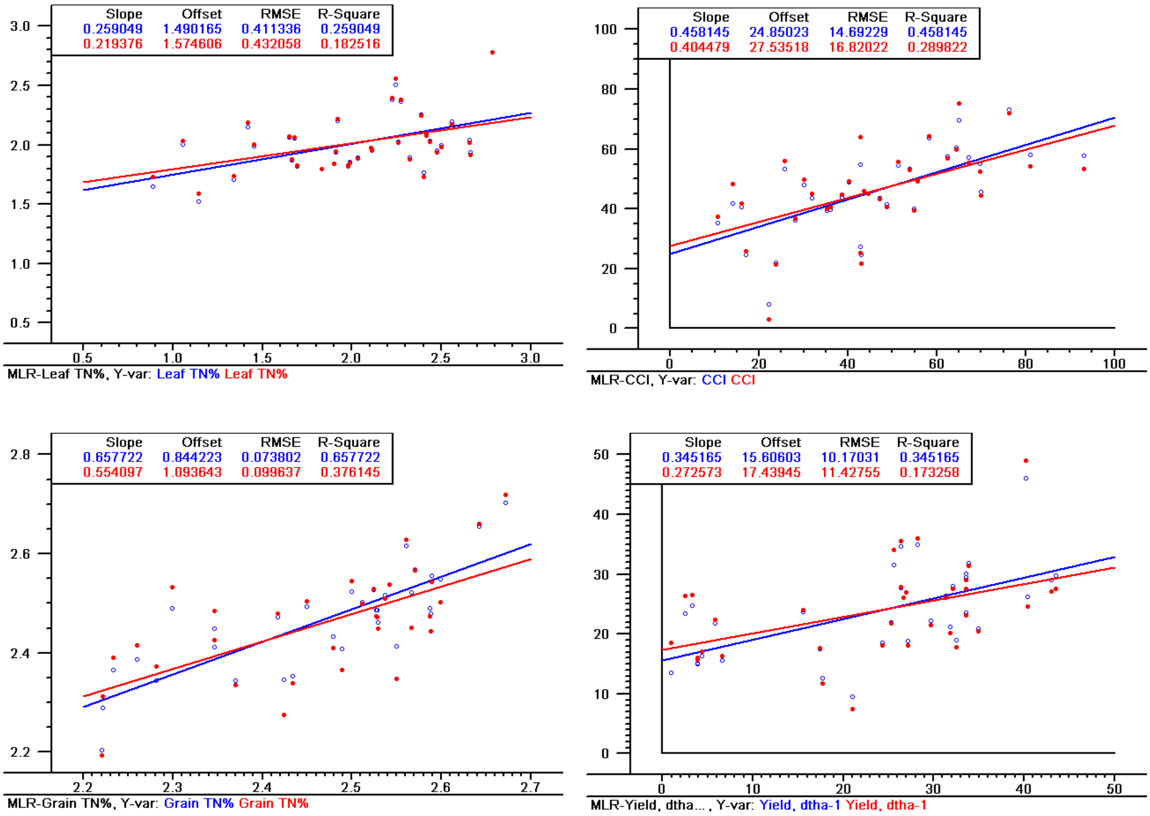


Figure 9.9 – Scatter plots of the MLR model performed using full cross validation method from 1st derivative of reflectance acquired at growth stage F10.5 - "Fiesta"/2008 showing relationship between predicted and observed winter wheat leaf TN content (%), CCI, grain TN content (%) and grain yield (dt ha^{-1}) (n=36). ● calibration ● validation

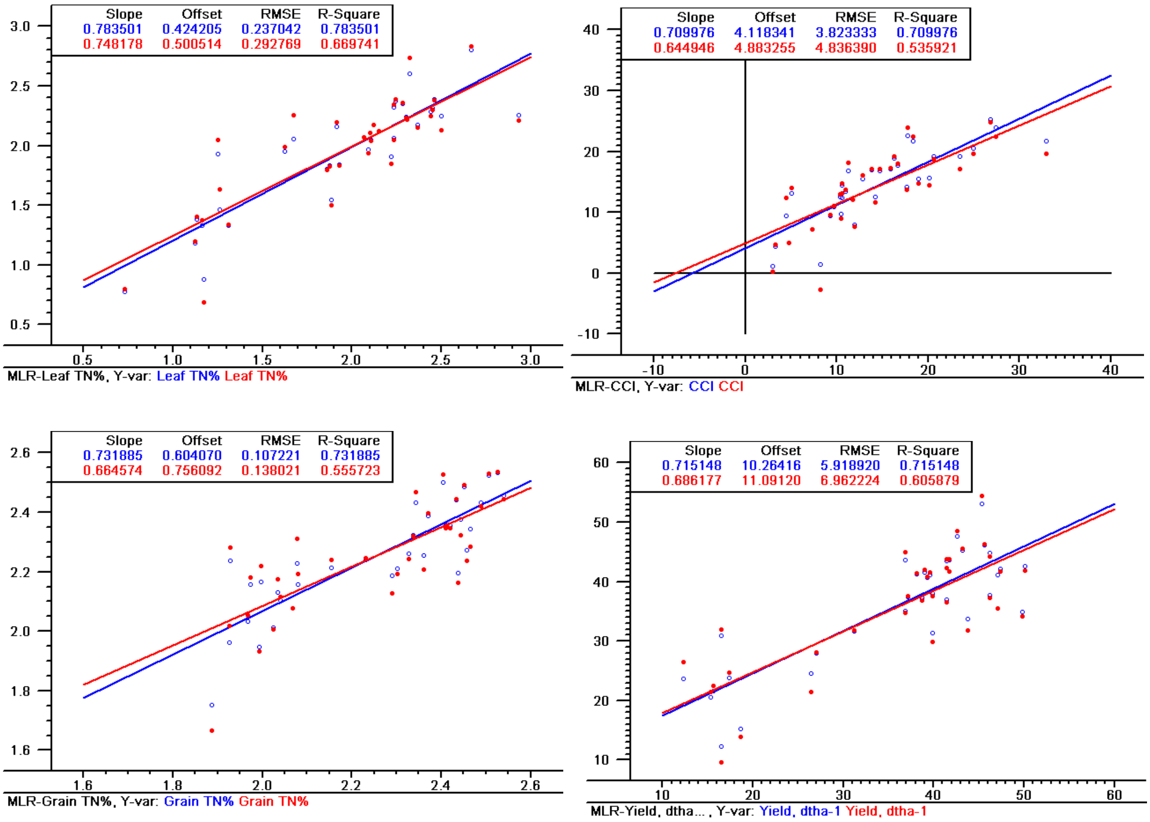


Figure 9.10 – Scatter plots of the MLR model performed using full cross validation method from 1st derivative of reflectance acquired at growth stage F10.5 - "Lucija"/2010 showing relationship between predicted and observed winter wheat leaf TN content (%), CCI, grain TN content (%) and grain yield (dt ha^{-1}) (n=36). ● calibration ● validation

Table 9.1. Means of Canonical Variables (PCA scores - 1st derivative of reflectance, F8 - “Lucija”/2010).

Group (N applied, kg ha^{-1})	Root 1	Root 2
I (Control, N_0)	14.64000	-1.70876
II (N_{100}, N_{150}, N_{200})	-0.88575	4.20446
III (N_{250}, N_{300})	-6.65569	-2.29897

Table 9.2. Prediction accuracy of fertilizer treatment group classification using canonical discriminant analysis - Classification Matrix (PCA scores - 1st derivative of reflectance) for growth stage F8, “Lucija”/2010.

Group (N applied, kg ha^{-1})	Predicted group membership			
	Percent	I	II	III
I (Control, N_0)	100	8	0	0
II (N_{100}, N_{150}, N_{200})	100	0	12	0
III (N_{250}, N_{300})	100	0	0	16
Total	100	8	12	16

Rows: Observed classifications; Columns: Predicted classification

Table 9.3. Predictions spreadsheet for fertilizer treatment group (PCA scores of 1st derivative of reflectance, F8 - "Lucija"/2010); Samples: Train, Test.

Case name	Sample	Treatments Target	Treatments - Output MLP 16-9-3	Treatments - Residuals MLP 16-9-3	Treatments - Confidence levels MLP 16-9-3
I-1	Test	I	I	Correct	0.999974
I-2	Train	I	I	Correct	1.000000
I-3	Test	I	I	Correct	1.000000
I-4	Train	I	I	Correct	1.000000
II-1	Train	I	I	Correct	1.000000
II-2	Test	I	I	Correct	1.000000
II-3	Test	I	I	Correct	0.999995
II-4	Test	I	I	Correct	1.000000
III-1	Train	II	II	Correct	0.999687
III-2	Test	II	II	Correct	0.510032
III-3	Train	II	II	Correct	0.999401
III-4	Train	II	II	Correct	0.999575
IV-1	Train	II	II	Correct	0.999049
IV-2	Test	II	II	Correct	0.829865
IV-3	Test	II	II	Correct	0.997949
IV-4	Train	II	II	Correct	0.999504
IX-1	Test	III	III	Correct	0.999996
IX-2	Train	III	III	Correct	1.000000
IX-3	Train	III	III	Correct	1.000000
IX-4	Train	III	III	Correct	1.000000
V-1	Test	II	III	Incorrect	0.968977
V-2	Test	II	II	Correct	0.625872
V-3	Test	II	II	Correct	0.528182
V-4	Train	II	II	Correct	0.996440
VI-1	Test	III	III	Correct	0.983992
VI-2	Test	III	III	Correct	0.999862
VI-3	Train	III	III	Correct	0.998637
VI-4	Train	III	III	Correct	0.999973
VII-1	Test	III	III	Correct	1.000000
VII-2	Train	III	III	Correct	1.000000
VII-3	Test	III	III	Correct	0.972595
VII-4	Train	III	III	Correct	0.998991
VIII-1	Train	III	III	Correct	0.999995
VIII-2	Test	III	III	Correct	0.984583
VIII-3	Test	III	III	Correct	0.937130
VIII-4	Train	III	III	Correct	0.998212

Table 9.4. Predictions spreadsheet for winter wheat yield (PCA scores of 1st derivative of reflectance, F8 - "Lucija"/2010); Samples: Train.

Case name	Yield Target	Yield - Output MLP 16-9-1	Yield - Residuals MLP 16-9-1	Yield - Std. Residuals MLP 16-9-1	Yield - Abs. Residuals MLP 16-9-1	Yield - Squared Residuals MLP 16-9-1
I-2	17.40000	14.85208	-2.54792	-0.68572	2.547921	6.49190
I-4	16.50000	17.01091	0.51091	0.13750	0.510907	0.26103
II-1	16.50000	15.58659	-0.91341	-0.24582	0.913408	0.83431
III-1	39.90000	46.38529	6.48529	1.74537	6.485292	42.05901
III-3	27.00000	26.45244	-0.54756	-0.14736	0.547563	0.29983
III-4	41.70000	38.83673	-2.86327	-0.77059	2.863271	8.19832
IV-1	47.10000	45.30099	-1.79901	-0.48416	1.799012	3.23644
IV-4	43.80000	46.57078	2.77078	0.74569	2.770784	7.67724
IX-2	41.40000	41.89910	0.49910	0.13432	0.499100	0.24910
IX-3	39.00000	43.20463	4.20463	1.13158	4.204633	17.67894
IX-4	31.20000	33.01021	1.81021	0.48718	1.810209	3.27686
V-4	41.40000	40.39111	-1.00889	-0.27152	1.008890	1.01786
VI-3	39.90000	40.82709	0.92709	0.24951	0.927092	0.85950
VI-4	38.70000	45.28365	6.58365	1.77184	6.583647	43.34440
VII-2	38.10000	38.16624	0.06624	0.01783	0.066238	0.00439
VII-4	50.10000	45.11299	-4.98701	-1.34214	4.987007	24.87024
VIII-1	46.20000	44.03507	-2.16493	-0.58264	2.164934	4.68694
VIII-4	45.30000	39.70414	-5.59586	-1.50600	5.595864	31.31369

Table 9.5. Predictions spreadsheet for winter wheat yield (PCA scores of 1st derivative of reflectance, F8 - "Lucija"/2010); Samples: Test.

Case name	Yield Target	Yield - Output MLP 16-9-1	Yield - Residuals MLP 16-9-1	Yield - Std. Residuals MLP 16-9-1	Yield - Abs. Residuals MLP 16-9-1	Yield- Squared Res. MLP 16-9-1
I-1	15.30000	21.73654	6.4365	1.73225	6.43654	41.4290
I-3	26.40000	16.03876	-10.3612	-2.78850	10.36124	107.3553
II-2	12.30000	13.79135	1.4914	0.40136	1.49135	2.2241
II-3	15.60000	14.83309	-0.7669	-0.20640	0.76691	0.5882
II-4	18.60000	14.18644	-4.4136	-1.18781	4.41356	19.4795
III-2	36.90000	27.75060	-9.1494	-2.46236	9.14940	83.7116
IV-2	42.60000	39.05473	-3.5453	-0.95413	3.54527	12.5689
IV-3	36.90000	34.28486	-2.6151	-0.70381	2.61514	6.8390
IX-1	45.60000	39.49571	-6.1043	-1.64283	6.10429	37.2624
V-1	49.80000	43.67770	-6.1223	-1.64768	6.12230	37.4826
V-2	41.40000	37.54815	-3.8519	-1.03664	3.85185	14.8368
V-3	39.60000	40.75205	1.1521	0.31005	1.15205	1.3272
VI-1	46.20000	39.45636	-6.7436	-1.81490	6.74364	45.4767
VI-2	37.20000	42.52719	5.3272	1.43370	5.32719	28.3790
VII-1	43.20000	39.04960	-4.1504	-1.11699	4.15040	17.2259
VII-3	47.40000	46.69356	-0.7064	-0.19012	0.70644	0.4991
VIII-2	39.30000	41.59979	2.2998	0.61894	2.29979	5.2890
VIII-3	41.70000	37.63390	-4.0661	-1.09430	4.06610	16.5332

10 INDEX

ANN - Artificial neural networks

ANOVA - Analysis of variance

CA – Correlation analysis

CCI – Chlorophyll content index

Chl – Chlorophyll

CLA – Cluster analysis

CV – Cross validation

DA - Discriminant analysis

EM – Electromagnetic (spectra)

F8 – Stem extension (Feekes` scale)

F10.5 - Heading (Feekes` scale)

MLR - Multiple linear regression

N – Nitrogen

NDVI – Normalized difference vegetation index

NUE – Nitrogen use efficiency

PCA - Principal component analysis

PC/PCs – Principal components

PLSR - Partial least square regression

r – Correlation coefficient

R - Reflectance

RMSEC – Root mean square error of calibration

RMSEP - Root mean square error of prediction

RS – Remote sensing

RVI – Simple ratio vegetation index

$\mathbf{R^2}$ – Coefficient of determination

SLR - Simple linear regression

TN – Total nitrogen

VI/VIs – Vegetation index/indices

λ – Wavelength

CURRICULUM VITAE

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Employment and work experience

2004 - now

Faculty of Agriculture, University of Zagreb, Department of General Agronomy

Teaching Assistant (PhD student); (identification number of scientist: 263291)

Teaching

Undergraduate study program (BSc) – Agroclimatology, Basics of Agriculture, Agriculture and Environment (Associate)

Graduate study program (MSc) - Global Ecology, Fertilizers and Fertilization (Associate)

Scientific interests

Agroecology, Remote sensing in agriculture – field spectroscopy and satellite imagery, Geographic information system (GIS), Precision agriculture, Crop yield simulation modeling

Training and research experience

2010 - Scientific training in domain of spectral data processing and biochemical modeling using ANN and multivariate statistics in collaboration with Professor Amy Kaleita, Iowa State University, Department of Agricultural and Biosystems Engineering, Ames, Iowa, SAD

2010 - EUFAR FP7 - N4EWG: Expert Working Groups - Hyperspectral Applications for Soil: An EUFAR Workshop on „QUANTITATIVE APPLICATIONS OF SOIL SPECTROSCOPY“, Potsdam, Germany

2008 - "International Short Course on Introduction to Spatial Data Processing", Faculty of Agriculture, Zagreb, Croatia

2008 - ASD "Chemometrics & Instrumentation Training", Analytical Spectral Devices Inc. (ASD.inc.), scientific training on remote sensing techniques in agriculture: field spectrometry with qualitative and quantitative spectral data analysis techniques, Boulder, Colorado, USA
2008 - Scientific education in domain of field remote sensing techniques in agriculture with implementing collaboration on joint supervision of my doctoral thesis with Professor Amy Kaleita, Iowa State University, Department of Agricultural and Biosystems Engineering, Ames, Iowa, SAD
2005 - "Advanced GIS modeling", scientific workshop on spatial data analysis, Faculty of Agriculture, Zagreb, Croatia
2005 - "Spatial data analysis with GIS" - MSc module, University of Hohenheim, Stuttgart, Germany, (TEMPUS program)

Current science projects

Nitrogen fertilization acceptable for environment (Project coordinator: Milan Mesić; 178-1780692-0695, funded by Ministry of Science, education and sports, MZOS) – associate
Agro-technical measures aimed at improving quality of organic products (Project coordinator: Ivica Kisić; 178-0672345-2767, funded by Ministry of Science, education and sports, MZOS) - associate

Current expert projects

Calcification with dolomite (Coordinator: Prof.dr.sc. Milan Mesić; Purchaser: Holcim mineral aggregates - associate

Other professional activities

2007 - Educational program: Forum for educational freedom "Active learning and critical judgment in higher education", workshop, Zagreb, Croatia
2005 till now - Member of: The Croatian Society of Soil Science (HTD)
http://www.tloznanstvo.hr/main.htm

Scientific publications:

Croatian Scientific Bibliography (CROSBI)

http://bib.irb.hr/lista-radova?autor=263291