Consequence of Diverse Nitrogen Levels on Leaf Pigments in Five Rice Genotypes Under Field Emergent Circumstances

Hukum Singh[1*], Amit Verma[2], M. Krishnamoorthy[3] and Alok Shukla[4]

[1,4]Department of Plant Physiology, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (263 145), India
[2]Department of Biochemistry, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (263 145), India
[3]Seed Technology Division, Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu (641 002), India

Article History

Manuscript No. 42
Received 30th October, 2010
Received in revised form 11th November, 2010
Accepted in final form 11th November, 2010

Correspondence to

*E-mail: hukumsingh97@yahoo.com

Keywords

Chlorophyll, carotenoids, nitrogen, active tillering stage

Abstract

The paper assesses the effect of dissimilar nitrogen levels on chlorophyll *a*, *b*, *a*/*b*, *a*+*b* and carotenoids content in assorted rice genotypes growing in field situation. The experiment was conducted throughout *kharif* season in 2008 and 2009. Five rice genotypes specifically KRH-2, Vasumati, Kasturi, Tulsi and Krishna Hamsa were taken as investigational material and urea was taken as a nitrogen source. The pigments content were anticipated at the instance of active tillering (AT) and flowering stage (FS). The pigments value was increased simultaneously with N levels and highest value was calculated at AT than FS. Chlorophyll *a*/*b* was increased after AT stage. Chlorophyll *a* was recorded higher for KRH-2 (AT) and Kasturi (FS), lower for Tulsi (AT) and Vasumati (FS). Chlorophyll *b* content was recorded greatest for Tulsi (AT) and Kasturi (FS) while minimum for Krishna Hamsa (AT) and Vasumati (FS). The upper limit of Chlorophyll *a*/*b* content was considered for Krishna Hamsa and Vasumati at AT and FS, although lower limit for Kasturi at same stage. Chlorophyll *a*+*b* content was calculated uppermost for KRH-2 (AT) and Kasturi (FS) whereas lowermost for Vasumati at both stages. Carotenoids contents were also drastically increased up to topmost N level and upper limit was recorded for KRH-2 and slighter for Vasumati at identical stage.

1. Introduction

Chlorophyll is the key molecule that captures the sunlight which is used in photosynthesis by green plants. Some factors affect chlorophyll content, for example, fertilizer management and its application time and type, plant density and light intensity in growth season, diseases and environmental stresses (Carter, 1998; Hussain et al., 2000; Moran et al., 2000). Carotenoids are efficient free-radical scavengers which absorb blue light. They serve two key roles in plants and algae. They absorb light energy for use in photosynthesis, and protect chlorophyll from photo damage (Armstrong and Hearst, 1996; Paiva and Russell, 1999). Generally, plant growth depends on nutrients supplied by photosynthesis and transported by water between important plant organs. Due to high incorporation of leaf nitrogen in chlorophyll, the nutrient status can be estimated indirectly by chlorophyll content. So, concentrations of leaf pigments could be used as an indicator of plant growth and development (Kozlowski et al., 1991; Filella et al., 1995; Moran et al., 2000). The N content of leaf is an important contribution to the photosynthetic rate and the photosynthetic nitrogen-use efficiency. Three factors important for photosynthetic function are chlorophyll content of leaf, chlorophyll *a*: *b* ratio, and total leaf N content (Hu et al., 2006). Leaf chlorophyll concentrations have a positive correlation with leaf N concentrations, but the changes in leaf chlorophyll may be smaller than the changes in leaf N. These studies exposed the dependence of leaf chlorophyll concentrations on leaf N and N fertilization (Boggs et al., 2003; Zhao et al., 2005a,b; Li et al., 2007; Erley et al., 2007; Wu et al., 2008). Along with chlorophylls, carotenoids also decrease with decrease in leaf N concentrations (Chandler and Dale, 1995; Zhao et al., 2005a,b). A positive relationship was found between leaf chlorophyll content of the selected leaf grown under low N supply in nutrient solution and grain yield under low N supply in the field experiments (Erley et al., 2007). Radin and Mauney (1986) reported that decrease in net photosynthesis rate due to N deficiency could be attributed to the decline in chlorophyll which is an important photosynthetic pigment. N deficiency reduces CO_2 assimilation and photosynthesis at light saturation levels. This can be due to the reduced enzyme (Rubisco) activity that in turn affects the functioning of the Calvin cycle (An and Shangguan, 2008; Jiang et al., 2004; DaMatta et al., 2002; Reddy et al., 1996). Zarco-Tejada et al. (2002) found that vegetation under stress is potentially indicated by the decline in chlorophyll pigments. The leaf chlorophyll pigments directly influence the light harvest and biogenesis of electron transport system for photosynthesis.

Therefore, the purpose of this study was to estimate and compare chlorophyll and carotenoids contents in five rice genotypes under different N availability in field growing condition

2. Materials and Methods

2.1. Study area

The trial was carried out at Dr. N. E. Borlaug's Crop Research Center, Department of Plant Physiology, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India during *kharif* season 2008 and 2009. Geographically, the site lies in Tarai plains about 30 km southwards of foothills of Shivalik range of the Himalayas at 29° N latitude, 79°29' E longitude and at an altitude of 243.8 msl. In Tarai region, the climate is humid and sub-tropical with hot summers to cool winters. Monsoon showers here in the mid of June up to the end of September.

2.2. Plant material

The seeds of five rice genotypes explicitly KRH-2 (hybrid), Kasturi, Krishna Hamsa, Tulsi and Vasumati (inbred) were used as investigational material. The seedlings were transplanted with 20 cm row space and 10 cm plant to plant distance. 200 mg of leaf material was collected from the second and third leaf of the shoot tip of each rice genotype at active tillering (AT) and flowering stage (FS).

2.3. Fertilizer application

The N (0, 50, 100 and 200 kg ha^{-1}) was applied in the form of urea fertilizer. The sub-plot size was 4 x 4 m^2. N was sprouted in three splits as 50% at 15 days after transplanting, 25% at panicle initiation and remaining 25% at FS.

2.4. Extraction and quantification of pigments

Concentration of chlorophyll *a*, *b*, and total chlorophyll was analyzed following the method of Arnon (1949). 200 mg of leaf sample was taken and ground using 10 ml of 80% acetone by mortar and pestle. Then the extract was transferred in a test tube sealed with parafilm to prevent evaporation and then stored in dark for 24 h. The chlorophyll *a*, *b*, and carotenoids content were measured using a UV-visible spectrophotometer (Systronics, India) at wavelengths 663, 645 and 470 nm. A solution of 80% acetone was used as a blank. The procedure given by Cha-um et al. (2004) was applied for estimation and calculation of carotenoids content. The chlorophyll *a*, *b*, *a*+*b*, and *a*/*b* content was expressed in mg g^{-1} fresh weight, and carotenoids in µg g^{-1} fresh weight in the leaf tissues.

2.5. Statistical analysis

The field experiment was laid out in a split plot design with three replicates. The data was analyzed statistically (Panse and Sukhatme, 1978) for calculating standard error of mean (SEm), and CD was taken at 5% for the comparison of chlorophyll and carotenoids contents in five rice genotypes under different N levels. Analyses of variance of the data are shown in Table 1, 2, 3, 4 and 5. The entire pigments were significantly enhanced by increasing N levels in all genotypes.

3. Results and Discussion

3.1. Chlorophyll content

The evaluated findings showed that the pigments content in leaf tissue were increased up to active tillering (AT) but decreased at flowering stage (FS) in all genotypes at all N levels. The chlorophyll *a* content was recorded maximum for rice genotype KRH-2 at both stages, while minimum was recorded for Tulsi at AT, and Vasumati at FS (Table 1). Similar results were obtained by Choubey and Choubey (2004) who found that chlorophyll *a* concentration was high during early growth stages (vegetative and early reproductive stages) and decreased during the flowering and maturity stage.The effect of N on chlorophyll *b* content was recorded at same stage and it was found that chlorophyll *b* also increased during AT and decreased at FS with the application of N from N_0 to N_{200} level. At AT stage, chlorophyll *b* content was calculated highest for Tulsi and the minimum for Krishna Hamsa, and thereafter at FS it was maximum for Kasturi and the minimum for Vasumati (Table 2). The evaluated results showed that the chlorophyll *a*+*b* content

Table 1: Effect of different N levels on chlorophyll *a* content at AT and FS of five rice genotypes

Genotype	Chlorophyll *a* content (mg g^{-1})									
	AT					FS				
	N_0	N_{50}	N_{100}	N_{200}	Mean	N_0	N_{50}	N_{100}	N_{200}	Mean
KRH-2	0.217	0.219	0.259	0.349	0.261	0.188	0.19	0.225	0.304	0.227
Vasumati	0.190	0.200	0.260	0.322	0.243	0.161	0.164	0.227	0.296	0.212
Kasturi	0.204	0.286	0.229	0.288	0.252	0.192	0.255	0.203	0.261	0.228
Tulsi	0.219	0.223	0.231	0.281	0.239	0.196	0.198	0.206	0.255	0.214
Krishna Hamsa	0.201	0.230	0.335	0.258	0.256	0.172	0.2	0.285	0.231	0.222
Mean	0.206	0.232	0.263	0.300		0.182	0.201	0.229	0.269	
	Treatment (T)		Variety (V)	T × V		Treatment (T)		Variety (V)	T × V	
SEm±	0.0020		0.0018	0.0037		0.0004	0.0006		0.0013	
CD (p=0.05)	0.0070		0.0054	0.0109		0.0014	0.0019		0.0039	

AT=Active tillering, FS=Flowering stage

was directly affected by N fertilizers. It was enhanced by the application of N fertilizer from N_0 to N_{200} level. The highest value of chlorophyll *a*+*b* was estimated for Kasturi at AT and KRH-2 at FS whiles lowest for Vasumati at both stage. (Table 3). Chlorophyll *a*/*b* content also increased at AT and FS with increase in N fertilizer from N_0 to N_{200} levels. The maximum chlorophyll *a*/*b* content at AT was found for rice genotype Krishna Hamsa and minimum for Kasturi. At FS, its upper limit was recorded in rice genotype Vasumati and the lowest in Kasturi (Table 4).

Chlorophyll *a*:*b* ratio was positively correlated with the net assimilation rate (NAR), nitrogen content plant^{-1}, and grain yield and crop growth period. It is also showed that there is linear relationship between leaf color chart values and Soil Plant Analysis Development (SPAD) values (Yang et al., 2003). Fertilizer management, application time, plant age, variety type, and environmental stresses are responsible for chlorophyll content. Similar results were found by several workers on positive relationship between N supply and the chlorophyll content of leaf. A non-limiting supply of N extends the life span of a leaf by delaying the onset of chlorophyll loss during grain filling (Yang et al., 2000; Evans, 1983; Seligman et al., 1983). Leaf chlorophyll concentration is directly related to leaf N concentration. The decline in leaf N concentration impairs chlorophyll pigment concentrations in the leaf. Under severe N stress conditions, the total chlorophyll concentrations get decreased by about 50% compared to plants (Ciompi et al., 1996). Zhao et al. (2005a,b) worked on the effects of N deficiency on leaf chlorophyll concentrations and found that with decrease in N fertilization, leaf N and leaf chlorophyll concentrations decreased simultaneously.

Table 2: Effect of different N levels on chlorophyll *b* content at AT and FS of five rice genotypes

Genotype	Chlorophyll *b* content (mg g^{-1})									
	AT					FS				
	N_0	N_{50}	N_{100}	N_{200}	Mean	N_0	N_{50}	N_{100}	N_{200}	Mean
KRH-2	0.135	0.153	0.180	0.234	0.176	0.098	0.111	0.123	0.167	0.125
Vasumati	0.176	0.119	0.147	0.239	0.170	0.077	0.087	0.100	0.174	0.110
Kasturi	0.170	0.201	0.172	0.219	0.191	0.103	0.154	0.134	0.161	0.138
Tulsi	0.191	0.141	0.190	0.278	0.200	0.093	0.105	0.142	0.205	0.136
Krishna Hamsa	0.200	0.140	0.156	0.170	0.166	0.098	0.110	0.112	0.130	0.113
Mean	0.174	0.151	0.169	0.228		0.094	0.113	0.122	0.167	
	Treatment (T)		Variety (V)	T × V		Treatment (T)		Variety (V)	T × V	
SEm±	0.0008		0.0009	0.0018		0.0009	0.0012		0.0024	
CD (p=0.05)	0.0028		0.0026	0.0052		0.0031	0.0036		0.0072	

AT=Active tillering, FS=Flowering stage

Table 3: Effect of different N levels on total chlorophyll content at AT and FS of five rice genotypes

Genotype	Total chlorophyll (*a*+*b*) content (mg g^{-1})									
	AT					FS				
	N_0	N_{50}	N_{100}	N_{200}	Mean	N_0	N_{50}	N_{100}	N_{200}	Mean
KRH-2	0.352	0.372	0.439	0.583	0.437	0.287	0.301	0.348	0.471	0.352
Vasumati	0.437	0.318	0.407	0.561	0.431	0.238	0.252	0.327	0.470	0.322
Kasturi	0.431	0.487	0.402	0.507	0.457	0.295	0.409	0.337	0.422	0.366
Tulsi	0.457	0.364	0.422	0.559	0.450	0.289	0.303	0.348	0.460	0.350
Krishna Hamsa	0.450	0.370	0.491	0.428	0.435	0.269	0.310	0.396	0.361	0.334
Mean	0.425	0.382	0.432	0.528		0.276	0.315	0.351	0.437	
	Treatment (T)		Variety (V)	T × V		Treatment (T)		Variety (V)	T × V	
SEm±	0.0023		0.0019	0.0039		0.0010	0.0014		0.0030	
CD (p=0.05)	0.0080		0.0057	0.0114		0.0035	0.0043		0.0086	

AT=Active tillering, FS=Flowering stage

3.2. Carotenoids content

Table 4: Effect of different N levels on chlorophyll *a*/*b* content at AT and FS of five rice genotypes										
Genotype	Chlorophyll *a*/*b* content (mg g^{-1})									
	AT					FS				
	N_0	N_{50}	N_{100}	N_{200}	Mean	N_0	N_{50}	N_{100}	N_{200}	Mean
KRH-2	1.612	1.437	1.439	1.493	1.495	1.915	1.714	1.824	1.820	1.818
Vasumati	1.633	1.682	1.773	1.345	1.608	2.092	1.882	2.262	1.699	1.984
Kasturi	1.407	1.421	1.330	1.316	1.369	1.854	1.659	1.519	1.619	1.663
Tulsi	1.782	1.575	1.215	1.012	1.396	2.110	1.879	1.454	1.250	1.673
Krishna Hamsa	1.631	1.647	2.142	1.520	1.735	1.757	1.828	2.549	1.786	1.980
Mean	1.613	1.552	1.580	1.337		1.946	1.792	1.922	1.635	
	Treatment (T)		Variety (V)	T × V		Treatment (T)		Variety (V)	T × V	
SEm±	0.013		0.017	0.034		0.015	0.017		0.035	
CD (*p*=0.05)	0.045		0.050	0.100		0.053	0.051		0.102	
AT=Active tillering, FS=Flowering stage										

The effect of different levels of N fertilizer on carotenoids content was predicted in all genotypes, and content was increased from N_0 to N_{200} level. At AT and FS, carotenoids content was calculated maximum for rice genotype KRH-2 and minimum for Vasumati (Table 5). Along with chlorophylls, other pigment contents like carotenoids also decline with decrease in leaf N concentrations (Zhao et al., 2005a,b; Chandler and Dale, 1995). It means carotenoids content is also increased with increasing N levels in leaf of rice plant. But some studies showed that decrease in N concentration resulted in an increase in carotenoids concentration (Zhao et al., 2003).

4. Conclusion

From the findings of this study it is concluded that the leaf pigment contents in all rice genotypes were enhanced by increasing N levels and vice-versa. This study will be useful in future for optimizing doses of N application in hybrid and inbred rice cultivation for better yield and output of rice plant because the leaf pigments directly influence the light harvest and biogenesis of electron transport system for photosynthesis. So, the selection and cultivation of high N efficient rice genotypes will provide the enough food to the increasing human population and will help to reduce the environmental pollution caused by N and its compounds such as ammonia, nitrites, nitrous oxide, and nitrates.

Table 5: Effect of different N levels on carotenoids content at AT and FS of five rice genotypes										
Genotype	Carotenoids content (µg g^{-1})									
	AT					FS				
	N_0	N_{50}	N_{100}	N_{200}	Mean	N_0	N_{50}	N_{100}	N_{200}	Mean
KRH-2	0.084	0.087	0.092	0.111	0.094	0.049	0.054	0.058	0.078	0.060
Vasumati	0.081	0.081	0.071	0.117	0.088	0.035	0.046	0.047	0.081	0.052
Kasturi	0.079	0.093	0.082	0.106	0.090	0.044	0.059	0.048	0.069	0.055
Tulsi	0.059	0.082	0.092	0.117	0.088	0.032	0.049	0.059	0.083	0.056
Krishna Hamsa	0.080	0.085	0.094	0.108	0.092	0.045	0.047	0.059	0.071	0.056
Mean	0.077	0.086	0.086	0.112		0.041	0.051	0.054	0.076	
	Treatment (T)		Variety (V)	T × V		Treatment (T)		Variety (V)	T × V	
SEm±	0.0009		0.0009	0.0018		0.0002	0.0006		0.0012	
CD (*p*=0.05)	0.0034		0.0026	0.0052		0.0009	0.0018		0.0037	
AT=Active tillering, FS=Flowering stage										

5. References

An, H., Shangguan, Z.P., 2008. Specific leaf area, leaf nitrogen content, and photosynthetic acclimation of *Trifolium repens* L. seedlings grown at different irradiances and nitrogen concentrations. Photosynthetica 46, 143-147.

Armstrong, G.A., Hearst, J.E., 1996. Carotenoids 2: genetics and molecular biology of carotenoids pigment biosynthesis. Journal of Federation of American Societies for Experimental Biology 10, 228-37.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts, polyphenoloxidases in *Beta vulgaris*. Plant Physiology 24, 1-15.
Boggs, J.L., Tsegaye, T.D., Coleman, T.L., Reddy, K.C., Fahsi, A., 2003. Relationship between hyperspectral reflectance, soil nitrate-nitrogen, cotton leaf chlorophyll and cotton yield: a step toward precision agriculture. Journal of Sustainable Agriculture 22, 5-16.
Carter, G.A., 1998. Reflectance wave bands and indices for remote estimation of photosynthesis and stomatal conductance in pine canopies. Remote Sensing of Environment 63, 61-71.
Chandler, J.W., Dale, J.E., 1995. Nitrogen deficiency and fertilization effects on needle growth and photosynthesis in sitka spruce (*Picea sitchensis*). Tree Physiology 15, 813-817.
Cha-um, S., Kirdmanee, C., Supaibulwatana, K., 2004. Biochemical and physiological responses of Thai jasmine rice (*Oryza sativa* L. ssp. Indica cv. KDML105) to salt stress. Science Asia 30, 247-253.
Choubey, V.K., Choubey, R., 2004. Spectral reflectance, growth and chlorophyll relationship for rice crop in semiarid region of India. Water Resources and Management 13, 73-84.
Ciompi, S., Gentilli, E., Guidi, L., Soldatini, G.F., 1996. The effect of nitrogen deficiency on leaf gas exchange and chlorophyll fluorescence parameters in sunflower. Plant Science 118, 177-184.
DaMatta, F.M., Loos, R.A., Silva, E.A., Loureiro, M.E., Ducatti, C., 2002. Effects of soil water deficit and nitrogen nutrition on water relations and photosynthesis of pot-grown *Coffea canephora* Pierre. Trees 16, 555-558.
Erley, S., Begum, N., Worker, M., Banziger, M., Horst, W. J., 2007. Leaf senescence induced by nitrogen deficiency as indicator of genotypic difference in nitrogen deficiency in tropical maize. Journal of Plant Nutrition and Soil Science 170, 106-114.
Evans, J.R., 1983. Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). Plant Physiology 72, 297-302.
Filella, I., Serrano, I., Serra, J., PeOuelas, J., 1995. Evaluating wheat nitrogen status with canopy reflectance indices and discriminant analysis. Crop Science 35, 1400-1405.
Hu, M.L., Wang, C.M., Yang, Q.H., Zhai, H.Q., Wan, J.M., 2006. QTL analysis for traits associated with photosynthetic function in rice (*Oryza sativa* L.). Research Notes III. Genetics of Physiological Traits and Others 21, 205-220.
Hussain, F., Bronson, K.F., Singh, Y., Singh, B., Peng, S., 2000. Use of chlorophyll meter for nitrogen management of irrigated rice in Asia. Agronomy Journal 92, 875-879.
Jiang, D., Dai, T., Jing, Q., Cao, W., Zhou, Q., Zhao, H., Fan, X., 2004. Effects of long-term fertilization on leaf photosynthetic characteristics and grain yield in winter wheat. Photosynthetica 42, 439-446.
Kozlowski, A., Petropoulou, Y., Manetas, Y., 1991. Summer survival of leaves in a soft-leaved shrub (*Phlomis fructicosa* L., Labiateae) under Mediterranean field condition: avoidance of photo inhibitory damage through decreased chlorophyll contents. Journal of Experimental Biology 46, 1825-1831.
Li, P., Caib, R., Gaoa, H., Penga, T., Wang, Z., 2007. Partitioning of excitation energy in two wheat cultivars with different grain protein contents grown under three nitrogen applications in the field. Physiologia Plantarum 129, 822-829.
Moran, J.A., Mitchell, A.K., Goodmanson, G., Stockburger, K.A., 2000. Differentiation among effects of nitrogen fertilization treatments on conifer seedling by foliar reflectance: a comparison of methods. Tree Physiology 20, 1113-1120.
Paiva, S.A., Russell, R.M., 1999. Beta-carotene and other carotenoids as antioxidants. Journal of the American College of Nutrition 18, 426-433.
Panse, V.G., Sukhatme, P.V., 1978. Statistical Methods for Agricultural Workers, IARI, New Delhi.
Radin, J.W., Mauney, J.R., 1986. The nitrogen stress syndrome. In: Mauney, J.R. and Stewart, J.M. (Eds.). Cotton Physiology. The Cotton Foundation, Memphis, TN, 91-105
Reddy, A.R., Reddy, K.R., Padjung, R., Hodges, H.F., 1996. Nitrogen nutrition and photosynthesis in the leaves of pima cotton. Journal of Plant Nutrition 19, 755-760.
Seligman, N.G., Loomis, R.S., Burke, J., Abshahi, A., 1983. Nitrogen nutrition and canopy temperature in field grown spring wheat. Journal of Agriculture Science 101, 691-697.
Wu, F.Z., Bao, W.K., Li, F.L., Wu, N., 2008. Effects of water stress and nitrogen supply on leaf gas exchange and fluorescence parameters of *Sophora davidii* seedlings. Photosynthetica 46, 40-48.
Yang, J., Zhang, J., Huang, Z., Zhu, Q., Wang, L., 2000. Remobilization of carbon reserves is improved by controlled soil-drying during grain filling of wheat. Crop Science 40, 1645-1655.
Yang, W.H., Peng, S., Huang, J., Sanico, A.L., Buresh, R.J., Witt, C., 2003. Using leaf color charts to estimate leaf nitrogen status of rice. Agronomy Journal 95, 212-217.
Zarco-Tejada, P.J., Miller, J.R., Mohammed, G.H., Noland, T.L., Sampson, P.H., 2002. Vegetation stress detection through chlorophyll a+b estimation and fluorescence effects on hyperspectral imagery. Journal of Environmental Quality 31, 1433-1441.
Zhao, D., Reddy, K.R., Kakani, V.G., Read, J.J., Carter, G.A., 2003. Corn (*Zea mays* L.) growth, leaf pigment concentration, photosynthesis and leaf hyperspectral reflectance properties as affected by nitrogen supply. Plant and Soil 257, 205-217.
Zhao, D., Reddy, K.R., Kakani, V.G., Reddy, V.R., 2005a. Nitrogen deficiency effects on plant growth, leaf photosynthesis, and hyperspectral reflectance properties of sorghum. European Journal of Agronomy 22, 391-403.
Zhao, D., Reddy, K.R., Kakani, V.G., Read, J.J., Koti, S., 2005b. Selection of optimum reflectance ratios for estimating leaf nitrogen and chlorophyll concentrations of field-grown cotton. Agronomy Journal 97, 89-98.