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Original Article

Genetic Analysis and Trait Association in Multi-Parent Advanced Generation Inter-Cross (MAGIC) F_2 Population of Tobacco (*Nicotiana tabacum* L.)

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Abstract

Genetic variability studies offers basic information related to genetic properties of the population based on which breeding methods could be formulated for further improvement of the crop. The estimates of heritability, coefficients of variability and genetic advance was calculated in a multi-parent advanced generation inter-cross (MAGIC) F_2 segregating populations for 10 traits including nicotine percentage, total soluble sugars and yield contributing traits during 2015 seasons at Northern Light Soils (NLS). The estimates of phenotypic coefficients of variation (PCV) were high and moderate for plant height (18.16%), number of leaves (15.96%), leaf area index (27.35%), internodal length (21.01%), stem diameter (16.73%), grade index (13.86%), nicotine percentage (19.87%), total soluble sugars (39.81%), chlorides (23.35%) and leaf yield (13.27%) in MAGIC-F_2 segregating populations at NLS 2015 season. High heritability coupled with high and moderate genetic advance was observed for all the traits studied. Correlation studies revealed that total soluble sugars was positively and significantly correlated with leaf area index, stem diameter, grade index and leaf yield. However total soluble sugars was negatively correlated with nicotine as total nicotine alkaloid and with chlorides percentage. Path coefficient analysis revealed that total percentage of sugars could be improved through selection of grade index, stem diameter, leaf area index and cured leaf yield which were positively correlated with total soluble sugar percentage. The current study also revealed that the MAGIC F_2 population could be used for development high sugar lines with moderate or low levels of nicotine and chlorides in tobacco.

Keywords: coefficients of variability, genetic advance, genetic bottleneck, genetic estimates, heritability

Introduction

Crop improvement depends largely on the genetic variability and implementation of possible breeding strategies for the selection of elite lines (Mohammadi and Prasanna, 2003). However the amount of variability available for the selection is relatively limited in majority of cultivated crops like wheat, maize, soybeans, chickpea, pigeon pea, lentil and cotton (Cooper *et al.*, 2001; Abdurakhmonov *et al.*, 2008) including model crop tobacco (Fricano *et al.*, 2012; Ganesh *et al.*, 2014; Chakravarthi *et al.*, 2017) due to various genetic bottle necks. Genetic bottlenecks imposed due to early domestication, predisposed selection and breeding activities and thus only a fraction of the variation carried to a cultivated variety from its gene pool. Moreover, extensive and repetitive use of superior genotypes with common ancestors also explained why the genetic base of the released varieties is narrow and the importance of the improvement of new varieties with essential variation (Fricano *et al.*, 2012; Basirnia *et al.*, 2014; Xaio *et al.*, 2015).

Tobacco (*Nicotiana tabacum* L., $2n = 48$) is one of the best model crop have also experienced a similar type of high inbreeding for several decades leading to a genetic bottleneck and limited divergence during its commercial cultivation of leaf (Fricano *et al.*, 2012; Ganesh *et al.*, 2014; Chakravarthi *et al.*, 2017). Tobacco leaf is not only used for making cigarettes but also used both in traditional and concurrent medicine in treating insect bites, cuts and tumours (Dadras *et al.*, 2014). More importantly, tobacco is an attractive green bioreactor proved to produce plant made vaccines, enzymes, immune modulatory molecules such as

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cytokines and high value pharmaceuticals (Phoolcharoen *et al*., 2011; Xaio *et al*., 2015). Despite the potential usage of tobacco in pharmaceutical and commercial production, limited cultivars exist with less harm associated and desirable traits. In order to develop such varieties, it is necessary to develop a population having more variability for yield and quality related traits (Chakravarthi *et al*., 2017). Recently, a multi-parent advanced generation intercross (MAGIC) strategy has been proposed to cross-examine genetic diversity (Cavanagh *et al*., 2008). MAGIC populations were first developed and described in Arabidopsis (Cavanagh *et al*., 2008; Kover *et al*., 2009; Huang *et al*., 2011). Cavanagh *et al*. (2008) discussed in detail the production of the MAGIC population, methods of mapping genes to traits and the relevance of such population to breeders. More recently, Huang *et al*. (2012) demonstrated the use of a large MAGIC wheat population to dissect out complex traits associations. Bandillo *et al*. (2013), revealed the increased recombination in MAGIC populations can lead to novel rearrangements of alleles and greater genotypic diversity. Given the potential benefits of MAGIC populations, the current study was aimed to develop MAGIC F_2 population to understand the genetic nature and magnitude of genetic variability, heritability, genetic advance, genetic divergence and traits associations using model crop tobacco.

Since yield and quality are inherited in a complex way and are influenced by the environment path coefficient analysis will be an added advantage to the breeder in crop improvement programme using segregating MAGIC F2 population. The study on above aspects is essential to identify and pick superior lines for trait development as they are likely to yield desirable recombinants in the progeny. Regarding this the genetic studies in tobacco were undertaken to estimate the genetic component of variance for yield and basic quality components of tobacco and calculate the heritability, coefficients of variability and genetic advance in MAGIC F2 segregating populations of the 8 crosses for 10 characters.

Materials and Methods

Seven exogenous Flue Cured Varieties (FCV) of *N. tabacum Viz*., PI-118133, PI-404973, PI-112162, PI-113441, PI-116084, PI-407474 and PI-552387 obtained from North Carolina State University, USA., were selected respectively for the different traits like number of leaves (30 ± 2), leaf area index (1,248 cm), internodal length (2.5 cm), stem diameter (8.5 cm), plant height (125 cm), total nicotine percentage (2.0%) and total soluble sugars (16%), along with commercial cultivar K326 consists of high curing quality (excellent), percentage of chlorides (0.5-1%) and leaf yield (2,500 kg/ha) for the development of MAGIC population. Each founding line was crossed to produce F_1s in (Northern light soils) NLS 2012 season and F_1s were inter-crossed in a half diallele manner to produce 28 IF_1s of two way cross hybrids. These were advanced in (Karnataka light soils) KLS 2013 season and selected 3 to 4 morphotypes, these were crossed in a half diallele manner and produced 420 nodes of four way cross hybrids. Obtained four way cross hybrids were advanced in NLS 2013 season and selected 3 to 4 morphotypes and inter-crossed again to produce roots of eight way cross hybrids. Total 20 roots were selected for advancement based on their pedigree information on manifestation of all eight combinations and advanced first time by selfing in NLS 2014. MAGIC F2 populations were advanced during NLS 2015 and data were collected from each segregated line and subjected to the genetic analysis to understand the existed variation in the population and the effects of nicotine and sugars with other agronomically important traits.

Evaluation of MAGIC F2 population

Data was recorded from total 248 MAGIC F_2 segregating independent individuals at maturity. Plant height was measured form soil surface to the tip of the buds. Total number of leaves per plant was recorded by counting the leaves of plants from the bottom to the tip of the main stock. Stem diameter and internodal length and leaf area measured at 5th, 10th and 15th leaf positions and mean values were used for the analysis. Leaf area (cm^2) was measured using length and width of the leaf and factored with 0.63 as proposed by Suggs *et al*. (1960).

To measure grade index, each leaf of the plant was harvested separately at maturity and cured in a barn. Cured leaves of each entry were graded based on the color, texture, body and aroma of the leaves and its positions and compared with total cured leaf weight. Total cured leaf weight of each plant was measured and calculated into kilogram per hectare and expressed in terms of leaf yield. To avoid any effect of plant position for basic chemistry analysis the 5th, 10th and 15th cured leaves of each plant was selected to obtain values of the total nicotine percentage and total soluble sugars and chloride percentages per plant.

Statistical analysis

The coefficient of variability both at phenotypic and genotypic levels for all the characters were computed by applying the formula as suggested by Burton and De Vane (1953). The genetic advance as percent of mean and heritability in broad sense for all the characters was estimated as the ratio of genotypic variance to the total phenotypic variance as suggested by Johnson and Rumbaugh (1995). Using correlation values, path coefficient analysis was done following the procedure of Dewey and Lu (1959). In path analysis, correlation coefficient is partitioned into direct and indirect effects of independent variable on the dependent variable by Akinnola (2012).

Results and Discussion

Genotypic coefficient of variation, phenotypic coefficient of variation, heritability in broad sense, genetic advance and mean genetic advance were estimated for basic chemistry and other yield related traits in MAGIC F_2 generation as presented in Table 1. Expectedly phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) in all the characters studied. The marginal difference between PCV and GCV is probably due to environmental effects. High heritability estimates for all the characters except moderate heritability for stem diameter suggesting that the environmental factors did not affect greatly the phenotypic performance of these traits. The highest PCV (39.81%) and GCV (39.00%) were observed for total soluble sugars percentage in plant. A similar finding of variation reported for total soluble sugars by Rehman and Qureshi (1997) and Shah *et al*. (2015) in tobacco.

A joint consideration of GCV, broad sense heritability and genetic advance revealed that plant height (17.63, 94.34 and 40.85%), leaf area index (26.09, 90.97 and 518.58%), Grade index (13.77, 98.68 and 19.45), leaf yield (13.15, 98.29 and 669.48%) and total soluble sugars plant^{-1} (39.00, 95.95 and 10.52%) combined high GCV, high broad sense heritability and moderate and high genetic advance. Thus, there is sufficient chance for improvement of these traits and selection based on the phenotypic performance will be reliable and effective from this MAGIC F_2 population. Furthermore, moderate to high heritability, GCV and GA% in a mean could be explained by additive gene action and their improvement could be achieved through mass selection in tobacco (Dobhal, 1987; Prasanna and Rao, 1989; Shah *et al*., 2008; Dadras *et al*., 2014) in other crops (Ogunbayo *et al*., 2014; Khatun *et al*., 2015; Hari *et al*., 2017).

Correlation coefficient analysis conducted in the current study revealed that total soluble sugars per plant was positively and significantly correlated with leaf area index, stem diameter, grade index and leaf yield (Table 2). These results suggest that selection to improve tobacco quality directed by phenotype of these traits may be effective. However total soluble sugars per plant was negatively significantly correlated with nicotine percentage as total nicotine alkaloids and chlorides revealing the possibility to improve tobacco quality with low nicotine and chlorides using current population. The observed positive correlation of total soluble sugars with various traits was supported by earlier workers (Maleki *et al*., 2011; Dadras *et al*., 2014). This indicates the relative utility of all these traits for selection with respect to sugar content and low nicotine.

The path analysis is a valuable tool to understand more clearly the association among different variables as recorded by simple correlation values. It helps to partition the overall association of particular variables with dependent variable into direct and indirect effects. While dealing with a more complex trait like total soluble sugars percentage, nicotine, chlorides and other yield traits, it enables the breeder to specifically identify the important component trait of such a nature and differential prominence can be laid on those characters for selection. Table 3 shows the results of the path analysis for the examined traits. Path coefficient analysis of basic chemistry traits and yield related traits revealed that grade index (0.29) had the highest positive direct effect on sugar content followed by stem diameter (0.12) showed direct effect on sugars. Internodal length and leaf yield had 5 and 6 percent direct effects respectively on sugar content. Hence, indirect selection based on grade index and stem diameter can adopted along with leaf yield and internodal length for enhancement of total sugars percentage and reduction of nicotine and chlorides as these traits showed negative significant correlation on total sugars.

Leaf area index showed a significant positive correlation but had negative direct effect with total soluble sugars may be because of negative indirect effects with plant height, number of leaves, nicotine percentage and chlorides may nullified the effect of leaf area index on total sugars. These conclusion is in accordance with Smalcelji (1998) and Ramachandra *et al*. (2015). They observed some traits failed to develop positive effects despite they had positive association due to nullifying action of certain traits on leaf yield via quality traits.

The results of the current study also revealed moderately high residual effect for genotypic (0.3364) path coefficients, indicating that all traits studied in the present investigation explained only about 66 percent of the genotypic variability via total soluble sugars percentage. Therefore, other attributes not included in this study are also contributing for total soluble sugars per plant (Table 3).

Conclusions

The present findings revealed that total percentage of sugars could be improved through selection of grade index, stem diameter, internodal length, leaf area index and leaf yield which were positively correlated with total soluble sugar percentage. Thus the current MAGIC F_2 population could be used for development high sugar lines with moderate or low levels of nicotine and chlorides. This study also reveals the successful usage of MAGIC population rather bi-parental populations for the development of lines with trait of our own interest and same strategy can be exploit to develop a varieties in other crops having low genetic diversity.

Table 1. Estimates of phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability of broad sense (h^2), Genetic advancement (GA) and percentage of GA Mean in MAGIC-F2 population

Trait	PCV	GCV	h^2	GA	GAM
Plant height (cm)	18.16	17.63	94.34	40.85	35.28
Number of leaves	15.96	14.63	84.09	7.33	27.65
Leaf area index	27.35	26.09	90.97	518.58	51.26
Internodal length (cm)	21.01	18.69	79.14	1.34	34.26
Stem diameter (cm)	16.73	13.16	61.87	1.39	21.33
Grade Index	13.86	13.77	98.68	19.45	28.17
Nicotine %	19.87	18.06	82.60	0.50	33.81
Total Sugars %	39.81	39.00	95.95	10.52	78.69
Chlorides %	23.35	22.23	90.67	0.85	43.60
Leaf yield (kg/ha)	13.27	13.15	98.29	669.48	26.86

Table 2. Phenotypic correlations among traits in Multi-parent Advanced Generation Inter Cross (MAGIC) F_2 generations

Trait	Total Sugars %	Plant height (cms)	Number of leaves	Leaf area index	Internodal length (cms)	Stem diameter (cms)	Grade Index	Nicotine %	Chlorides %	Leaf yield (kg/ha)
Total Sugars %	1.000									
Plant height (cm)	0.047	1.000								
Number of leaves	-0.038	0.531**	1.000							
Leaf area index	0.181**	0.410**	0.257**	1.000						
Internodal length(cm)	0.114	0.110	-0.198**	0.137*	1.000					
Stem diameter (cm)	0.236**	0.239**	0.118	0.704**	0.223**	1.000				
Grade Index	0.349**	0.272**	0.085	0.576**	0.174**	0.414**	1.000			
Nicotine %	-0.170**	0.151*	0.086	0.142*	0.056	0.086	0.051	1.000		
Chlorides %	-0.381**	-0.042	-0.003	0.063	0.032	-0.051	0.027	0.249**	1.000	
Leaf yield (kg/ha)	0.326**	0.351**	0.212**	0.553**	0.118	0.384**	0.898**	0.049	-0.011	1.000

*Significant at P≤0.05; **Significant at P≤ 0.01

Table 3. Genotypic direct (diagonal) and indirect effects of different quantitative traits in Multi-parent Advanced Generation Inter Cross (MAGIC) F_2 in response to the total sugars

Traits	Plant height (cm)	Number of leaves	Leaf area index	Internodal length (cm)	Stem diameter (cm)	Grade Index	Nicotine Percent	Chlorides percent	Leaf yield (Kg/ha)	r coefficients
Plant height (cm)	-0.053	-0.028	-0.022	-0.006	-0.013	-0.014	-0.008	0.002	-0.019	0.047
Number of leaves	-0.017	-0.033	-0.008	0.006	-0.004	-0.003	-0.003	0.000	-0.007	-0.038
Leaf area index	-0.019	-0.012	-0.045	-0.006	-0.032	-0.026	-0.006	-0.003	-0.025	0.181
Internodal length(cm)	0.006	-0.010	0.007	0.052	0.012	0.009	0.003	0.002	0.006	0.114
Stem diameter (cm)	0.028	0.014	0.083	0.026	0.117	0.049	0.010	-0.006	0.045	0.236
Grade Index	0.079	0.025	0.168	0.051	0.121	0.292	0.015	0.008	0.262	0.349
Nicotine %	-0.014	-0.008	-0.013	-0.005	-0.008	-0.005	-0.095	-0.023	-0.005	-0.170
Chlorides %	0.015	0.001	-0.023	-0.011	0.018	-0.010	-0.089	-0.360	0.004	-0.381
Leaf yield (kg/ha)	0.022	0.014	0.035	0.008	0.025	0.057	0.003	-0.001	0.064	0.326
Total indirect effects	0.100	-0.005	0.227	0.062	0.118	0.057	-0.076	-0.021	0.262	

Residual effect: 0.3364

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