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DEGREE OF ASSOCIATION AND CAUSE-EFFECT ANALYSIS FOR SPOT BLOTCH DISEASE RESISTANCE AND ANCILLARY CHARACTERS OF YIELD IN BARLEY (*HORDEUM VULGARE* L.)

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Abstract

Ninety-six genotypes of Barley were tested under natural as well as epiphytotically created biotic stress condition for spot blotch disease caused by *Bipolaris sorokiniana* (Sacc.) Shoemaker at Agriculture Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi which is a hot spot for spot blotch. The objective of this experiment was to know the nature of the association between pairs of characters associated with disease-resistant in spot blotch and seed yield at the phenotypic level and thereby compare the direct and indirect effect of character under study. Phenotypic correlation revealed that under natural condition association estimates have shown AUDPC has a significant negative relationship with grain yield (r = -0.46) followed by thousand-grain weight (r = -0.233), days to maturity (-0.168). Whereas, under biotic stress condition also it has significant and negative relationship with important yield and yield attributing traits viz. grain yield g/plant (r = -0.65) followed by days to maturity (r = -0.43), thousand grain weight (r = -0.27), grain filling duration (r = -0.23) and spike number per plant (r = -0.23). Thus implies that indirect selection for these traits helps in the development of spot blotch resistant genotypes. From the path coefficient analysis based on correlations, it was observed that under natural conditions maximum direct negative effect on AUDPC was exhibited by grain yield per plant (-0.478). However, the direct positive effect on AUDPC was observed for grain filling duration (0.030) and plant height (0.120) while under biotic stress condition maximum direct negative effect on AUDPC was exhibited by grain yield per plant (-0.589) followed by days to maturity (-0.381). Therefore, the characters which show negative direct effect may play a significant role in the formation of selection criteria for breeding the resistant genotypes. Thus, direct selection for these traits in order to achieve yield improvement will be fruitful. Thus, these characters play an important role in the formation of selection criteria to enhance the resistance to spot blotch in Barley. The present investigation suggests that selection in Barley germplasm based on the degree of association and causes effect analysis will be effective in selecting superior plants for yield parameters and spot blotch resistance in isolating high yielding disease resistant genotype in Barley.

***Key words**:* Spot blotch, *Bipolaris sorokiniana,* Area under disease progress curve, correlation, path analysis.

Introduction

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops in India and grown in about 100 countries worldwide (Prasad, 1992). During 2014, globally barley occupied the fourth rank amongst the cereal crops with 144.33 million ton production, after maize, rice and wheat (Kumar *et al.*, 2014) and share about 7% of the global cereal production (Pal *et al.*, 2012). It is frequently being described as the "*most cosmopolitan of the crops*" and also considered as *"crop of marginal farmers"* because of its low input requirement and better adaptability to harsh environments like drought, salinity, alkalinity and marginal lands (Kumar *et al.*, 2014).

However, in North-eastern plain zone barley crop is adversely threatened by leaf blight or spot blotch disease caused by *Bipolaris sorokiniana* pathogen. Many researchers have reported a range of yield loss in south Asia and India to be 19.6% and 15.5% respectively by Dubin & van Ginkal, 1991. Duveiller & Gilchrist, 1994 also reported 20 to 80% yield losses and if the infection is so much severe then up to 100% yield losses also

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reported from Srivastava *et al.*, 1971).

There is two major cause of correlation in genetics, i.e. pleiotropism and linkage disequilibrium (Allard, 1960). Pleiotropism will assist in explaining the degree of correlation among traits which are influenced by the same gene; in case of positive correlation, genes will increase both the characters, while negative correlation means one character value will increase while other will reduce (Hailu *et al.,* 2016). Association between characters can be easily assessed by correlating the phenotypic values and this value can be measured in a number of individuals (Falconer & Mackay, 1996).

To correlate the variable, the correlation coefficient is measured which is absolute value between characters under study. It will not tell about the cause and effect relationship (Roy, 2000). Path coefficient analysis is an important statistical tool to intimate that which variable (causes) exert influence on other variable (effects), while recognizing the effect of multi-colinearity. (Akanda and Mundt, 1996). The direct influence of one variable upon other variable is measured by path coefficient and separates the correlation coefficient into components of direct and indirect effects. The correlation will only measure mutual association without considering causation, while path coefficient analysis will consider the cause and measure the relative importance of characters (Dewey & Lu, 1959).

In any crop improvement program of a complex trait like yield, the direct selection is not effective, therefore it becomes essential to assess the contribution of each character and partitioning the correlation into components of direct and indirect effect (Giriraji & Vijayakumar, 1974).

In order to have resistance in barley genotypes against most damaging fungal disease “spot blotch’’ has a good promise to improve grain yield average and total barley production in the country. Hence, breeding for disease resistance will require the knowledge of component traits associated with yield and disease resistance (Prasad *et al*., 2013) in addition to pre-requisition of genetic variability for crop improvement (Chandra *et al*., 2016).

Therefore the objective of the present study was to estimate the association and contribution of component traits by comparing the direct and indirect effect of the character to breed for better yield & disease resistance towards spot blotch disease in Barley.

Materials and methods

Experimental site and season:

The experimental material for present investigation comprised of Ninety-six genotypes of Barley collected from several genotypes grown and maintained by All India Co-ordinated Barley Improvement Project. The experiment was conducted during the *rabi* (winter) season of 2016-17 at the Agriculture Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi as a hot spot for spot blotch screening located geographically at 25.28^{0}N latitude and 82.95^{0}E longitude in North Gangetic plain in the eastern part of Uttar Pradesh, India.

Data collected:

Data were collected for the following parameters like days to fifty percent flowering (when the plot had 50% heading on main spike number, days to maturity (counted as the number of days from sowing until the grains were completely hard and possessed moisture levels less than 12% at growth stage 92), Plant height (individual plant was measured in centimeters from the ground level to the tip of terminal spikelet (excluding the awn) of the main shoot at the dough stage *i.e* Zadoks growth stage 87 (Zadoks *et al*., 1974), Spike length with awns and without awns (length of main spike (cm) was measured from the base to the tip of the terminal spikelet, including the awns and excluding awn respectively, number of spike per plant (at the physiological maturity, the total number of spike bearing tillers in each plant was recorded), Grain filling duration (the observed grain filling period was determined by subtracting the time to maturity from the time to anthesis as per Duguid and Brule-Babel, 1994), Thousand grain weight (One thousand threshed grains were taken randomly after sun drying at 12% moisture level and weighted in gram with the help of electric balance), Grain yield per plant (the total weight of filled grains of each plant in grams was recorded after threshing).

Disease assessment

The spore suspension was prepared from 15 days old culture of *Bipolaris sorokiniana* multiplied on sorghum seeds (Joshi *et al*., 1969) and was inoculated at tillering stage, flag leaf stage and anthesis stages on Zadok scale crop growth stages (Zadoks *et al*., 1974) during evening hours following the method of Chaurasia *et al*., 1999. Plots were irrigated immediately after inoculation to maintain high relative humidity for facilitating disease establishment and development in the field. The three individual disease score was taken at weekly intervals using the double-digit scale (00-99) developed as a modifications of Saari and Prescott’s, 1975 at three different growth stages (GS), *viz*., GS 63 (beginning of anthesis to half-complete), GS 69 (anthesis complete) and GS 77 (late milking). Percentage of disease severity is estimated based on the following formula given by Sharma and Duveiller, 2007.

% of Disease Severity = (D1/9) × (D2/9) × 100

Where D1 refers to the first digit of double-digit, and D2 refers to the second digit of double-digit, Calculate the area under the disease progress curve (AUDPC) based on disease severity at GS63, GS69 and GS77 using the percent severity estimations corresponding to the disease ratings (Roelfs *et al.*, 1992).

$$\text{AUDPC} = \sum_{i=1}^{n} [\{(Yi + Y(i+1))/2\} \times (t(i+1) - ti)]$$

Where, Yi = disease level at time ti

t (i + 1) - ti = Time (days) between two disease scores

n = number of dates on which spot blotch was recorded

Statistical Analysis

Correlation coefficient analysis was done according to Robinson *et al.*, 1951 and the methodology proposed as suggested by Wright, 1921 and illustrated by Dewey and Lu, 1959 was followed to carry out path analysis for spot blotch AUDPC and its components keeping AUDPC as dependent variable and other parameters as independent variables. The analysis was done by using the Windostat 9.3 from Indostat series software.

$$r_{p(x,y)} \frac{Cov_p(x,y)}{\sqrt{\sigma^2_{px} . \sigma^2_{py}}}$$

Where,

r_p = Phenotypic correlation

Cov_p (x,y) = Phenotypic covariance between the characters x and y.

σ^2_{px} and σ^2_{py} are the phenotypic variances of the characters x and y.

The calculated value of r was compared with ‘r’ table value with (n – 2) degree of freedom at 5 and 1 percent level of significance, where n refers to a number of pairs of observation.

Standard path coefficients or standardized partial regression coefficients were obtained by solving the following set of ‘p’ simultaneous equations

$$P_{01} + P_{02}\, r_{12} + \ldots\ldots\ldots\ldots\ldots\ldots\ldots\; P_{0p}\, r_{1p} = r_{01}$$

$$P_{01}\, r_{21} + P_{02}\, r_{22} + \ldots\ldots\ldots\ldots\ldots\ldots\; P_{0p}\, r_{\,2p} = r_{02}$$

$$P_{0p}\, r_{1p} + P_{0p}\, r_{2p} + \ldots\ldots\ldots\downarrow\ldots\ldots\ldots P_{0p} = r_{0p}$$

Where,

P_{01}, P_{02}, P_{0p} are the direct path coefficients of variables 1, 2, 3…… p on the dependent variable 0.

r_{12}, r_{13} …. Pr_{1p}…… $r_{r(p-1)}$ are the possible correlation coefficients between various independent variables and r_{01}, r_{02} …. r_{0p} are the correlations between dependent and independent variables. The indirect effects of the ith variable via jth variable were obtained P_{oj} x r_{ij}.

The contribution of the remaining unknown factors was measured as the residual factor and calculated as below:

$$P^2_{ox} = 1 - (P^2_{01} + 2P_{01}P_{02}r_{12} + 2P_0P_{03}r_{13} + \ldots\ldots\ldots + P^2_{02} + 2P_{02}P_{03}r_{13} + \ldots\ldots\ldots\ldots + P^2_{0p})$$

Residual factor = $\sqrt{P^2_{OX}}$

Results and discussion

Yield is a very complex character governed by many genes, therefore, we need to identify those characters which can be easily observed and have a significant effect on yield. The present correlation studies give an insight into the degree of relationship among traits, particularly spot blotch disease components and yield contributing traits. This will ultimately help in further crop improvement programs in selection.

The correlation coefficients among different characters were worked out and presented in Table1 which reveal significant association of seed yield per plant under natural conditions (values shown above diagonal) of seed yield per plant was observed with the thousand-grain weight (r = 0.25) followed by grain filling duration (r = 0.16) and days to maturity (r = 0.14). These findings also represented through shaded correlation matrix in Figure 1. While under biotic stress condition (values shown below diagonal in table 1) also significant relationship with thousand-grain weight (r = 0.35) followed by grain filling duration (r = 0.31), days to maturity (r = 0.25), number of spike per plant (r = 0.17) at 1% level of significance and plant height (r = 0.14) at 5% level of significance. These findings also represented in shaded correlation matrix through Fig. 2. Which give an impression that making an indirect selection for these traits in crop improvement programs will substantially increase the yield level. These findings are in harmony with the results reported from Hailu *et al.*, 2016, Kole, 2006, Khodarahmpour *et al.*, 2011 and Olfati *et al.*, 2010.

Under natural condition, association estimates have shown that AUDPC has a significant negative relationship with grain yield (r = -0.46) followed by thousand-grain weight (r = -0.233), days to maturity (-0.168). Whereas, under biotic stress condition also it has significant and negative relationship with important yield and yield attributing traits viz. grain yield g/plant (r = -0.65) followed by days to maturity (r = -0.43), thousand grain weight (r = -0.27), grain filling duration (r = -0.23) and spike number per plant (r = -0.23).

The phenotypic correlation between plant height, days to 50% flowering, spike length with and without awns; and AUDPC values were non-significant. A similar finding was reported by Prasad *et al.*, 2013 for plant height.

Exhibiting a significant and negative association of Area under disease progress curve with different traits revealed that if we want to restrict the quantitative growth of spot blotch disease, then we may look on these negatively associated traits in terms of high yielding varieties, late-maturing genotypes, seeds with greater test weight, enhanced grain filling duration and more number of effective tillers.

However, research findings of Chethana *et al.*, 2018, Meena *et al.*, 2014, Virendra *et al.*, 2015 and Singh *et al.*, 2016 state that, days to maturity is negatively associated with seed yield so in this case, they are suggesting to use early maturing varieties. As per Chaurasia *et al.*, 2000 severity of spot blotch disease will increase as per growth advancement of the crop. However Shrestha *et al.*, 1998 reported that late-maturing varieties will get less disease. Duveiller *et al.*, 1998 reported that association between plant height, days to maturity and disease severity is complex. Joshi *et al.*, 2002, reported that genetic association between resistant plant and taller plant height and late maturity is not always true and it is possible to find the plant which is resistant but they are short and early maturing.

Reason behind the severity of spot blotch as the advancement of growth stage is pathogen (*Bipolaris sorokiniana*) is a weak parasite (Duveiller & Gilchrist, 1994) hence first of all it will colonize the older leavers which are close to the ground or the tissue which is stress and we know severity of any disease is increases by increase in amount of inoculums and in case of spot blotch the inoculum is again depended on amount of senescent leaf tissue available for growth and development of *Bipolaris sorokiniana* and Brandle *et al.*, 1987; Raemaekers, 1998 also reported that leaf senescence and spot blotch progress form lower leaf to upper leaf. Joshi *et al.*, 2002 also reported that disease expression is associated with growth stage irrespective of days to maturity, plant height, and other phonological characters.

There are similar findings as well as contrary findings from researchers with respect to the negative association of AUDPC to days to maturity. There is a need to study the spot blotch severity by taking checks of different maturing genotypes (Duveiller *et al.*, 1998).

Association of various plant traits with the trait of major and economic importance like seed yield is the consequence of their direct and indirect effects. Therefore, it becomes imperative to partition such

Table 1: Correlation coefficient under Natural condition (above diagonal) and under biotic stress condition (below diagonal).

Parameters	PH	DFF	GFD	SLWOA	SLWA	SPP	DM	GY	TGW
AUDPC	0.018	-0.090	-0.0657	-0.073	-0.105	-0.153	-0.168	-0.461	-0.233
PH		-0.220**	0.148*	0.350**	0.450**	0.130*	-0.046	0.227**	0.059
DFF	-0.06		-0.591**	0.083	-0.120*	-0.089	0.309**	-0.214**	-0.042
GFD	0.15*	-0.75**		-0.031	0.113	0.076	0.585**	0.305**	0.158**
SLWOA	0.28**	-0.16**	0.07		0.453**	0.034	0.047	-0.013	0.019
SLWA	0.29**	-0.05	0.02	0.22**		0.062	0.012	0.212**	0.092
SPP	-0.02	-0.06	0.14*	0.02	0.05		-0.0002	0.245**	0.053
DM	0.12*	0.33**	0.37**	-0.12*	-0.03	0.12*		0.145*	0.144*
GY	0.14*	-0.14*	0.31**	0.09	0.04	0.17**	0.25**		0.249**
TGW	0.31**	-0.19**	0.35**	0.04	0.16**	0.1	0.22**	0.35**	
AUDPC	0.01	-0.07	-0.23	-0.05	0.03	-0.23	-0.43	-0.65	-0.27

Under Natural condition - Significance Levels at 0.05* and 0.01** probability level If correlation r => 0.115, 0.152 respectively, by taking AUDPC as dependant variable.

Under biotic stress condition -Significance Levels at 0.05* and 0.01** probability level If correlation r => 0.12, 0.15 respectively, by taking AUDPC as dependant variable.

Where, PH = Plant height, D50% F= days to 50% flowering, DM= days to maturity, GFD= grain filling duration, SLWOA= spike length without awns, SLWA = spike length with awns, SPP = spikes per plant, GY= grain yield, TGW= thousand grain weight, AUDPC= Area under disease progress curve.

Table 2: Path matrix of Area under Disease Progress Curve in case of Natural condition.

Parameters	PH	DFF	GFD	SLWOA	SLWA	SPP	DM	GY	TGW
PH	0.155	-0.034	0.023	0.054	0.070	0.020	-0.007	0.035	0.009
DFF	0.029	-0.132	0.078	-0.011	0.016	0.012	-0.041	0.028	0.006
GFD	0.005	-0.018	0.030	-0.001	0.003	0.002	0.018	0.009	0.005
SLWOA	-0.035	-0.008	0.003	-0.100	-0.045	-0.003	-0.005	0.001	-0.002
SLWA	-0.015	0.004	-0.004	-0.015	-0.033	-0.002	0.000	-0.007	-0.003
SPP	-0.008	0.005	-0.005	-0.002	-0.004	-0.059	0.000	-0.014	-0.003
DM	0.002	-0.014	-0.027	-0.002	-0.001	0.000	-0.046	-0.007	-0.007
GY	-0.108	0.102	-0.146	0.006	-0.101	-0.117	-0.069	-0.478	-0.119
TGW	-0.007	0.005	-0.019	-0.002	-0.011	-0.006	-0.017	-0.030	-0.119
AUDPC	0.018	-0.090	-0.066	-0.073	-0.105	-0.153	-0.168	-0.461	-0.233
Partial R^2	0.003	0.012	-0.002	0.007	0.003	0.009	0.008	0.220	0.028

R^2 =0.288, Residual effect = 0.844 by taking AUDPC as dependent variable
Where, PH = Plant height, D50% F= days to 50% flowering, DM= days to maturity, GFD= grain filling duration, SLWOA= spike length without awns, SLWA = spike length with awns, SPP = spikes per plant, GY= grain yield, TGW= thousand grain weight, AUDPC= Area under disease progress curve.

Table 3: Path matrix of Area Under Disease Progress Curve in case of biotic stress.

Parameters	PH	DFF	GFD	SLWOA	SLWA	SPP	DM	GY	TGW
PH	0.120	-0.008	0.017	0.033	0.035	-0.002	0.014	0.016	0.037
DFF	-0.008	0.121	-0.091	-0.019	-0.006	-0.007	0.040	-0.017	-0.024
GFD	0.030	-0.153	0.203	0.015	0.005	0.029	0.076	0.064	0.071
SLWOA	0.009	-0.005	0.002	0.031	0.007	0.001	-0.004	0.003	0.001
SLWA	0.004	-0.001	0.000	0.003	0.012	0.001	0.000	0.001	0.002
SPP	0.002	0.006	-0.014	-0.002	-0.005	-0.098	-0.012	-0.017	-0.010
DM	-0.046	-0.125	-0.142	0.046	0.013	-0.045	-0.381	-0.096	-0.086
GY	-0.080	0.083	-0.186	-0.052	-0.024	-0.099	-0.149	-0.589	-0.206
TGW	-0.016	0.010	-0.018	-0.002	-0.009	-0.005	-0.012	-0.018	-0.052
AUDPC	0.014	-0.071	-0.228	0.051	0.029	-0.227	-0.427	-0.654	-0.266
Partial R^2	0.002	-0.009	-0.046	0.002	0.000	0.022	0.163	0.385	0.014

R^2 =0.533, Residual effect = 0.683
Where, PH = Plant height, D50% F= days to 50% flowering, DM= days to maturity, GFD= grain filling duration, SLWOA= spike length without awns, SLWA = spike length with awns, SPP = spikes per plant, GY= grain yield, TGW= thousand grain weight, AUDPC= Area under disease progress curve.

associations into measures of direct and indirect effects of component traits through path coefficient analysis. Thus it will help in determining the cause of association among traits. The path coefficient analysis helps in indirect selection for genetic improvement of yield because of its low heritability direct selection would not be very effective for yield improvement.

Path co-efficient analysis of AUDPC under natural conditions from Table 2 and Fig. 3 revealed that the maximum direct negative effect on AUDPC was exhibited by grain yield per plant (-0.478) followed by days to fifty percent flowering (-0.132) and thousand-grain weight (-0.119) and a number of spike per plant (-0.098). However, the direct positive effect on AUDPC was observed for grain filling duration (0.030) and plant height (0.120). Information on the association of yield attributes and spot blotch resistance and their direct and indirect effect on AUDPC in case of biotic stress were represented in Table 3 and Fig. 4. Path coefficient analysis revealed that the maximum direct negative effect on AUDPC was exhibited by grain yield per plant (-0.589) followed by days to maturity (-0.381), thousand-grain weight (-0.052) and a number of spike per plant (-0.098). Therefore, the characters which show negative direct effect may play a significant role in the formation of selection criteria for breeding the resistant genotypes. Thus, direct selection for these traits in order to achieve yield improvement will be fruitful. However, the direct positive effect on AUDPC was observed for grain filling duration (0.203), days to 50% flowering (0.121), plant height (0.120), spike length without awns (0.031) and spike length with awns (0.012).

All the direct effects were less than one which indicates that influences due to multicollinearity were minimal (Gravois and Helms, 1992). In the present

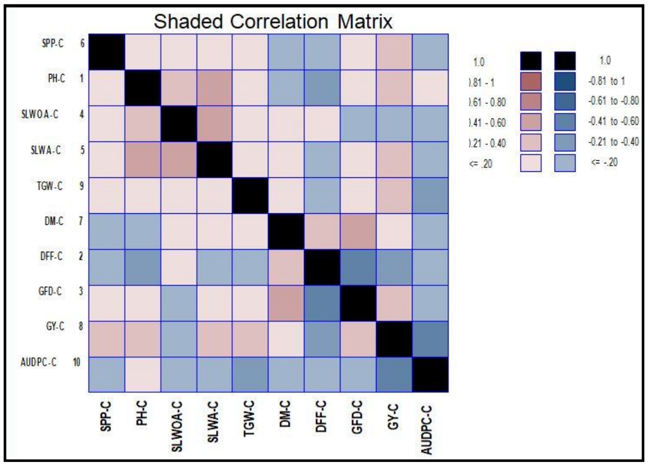

Fig. 1: Degree of association analysis matrix under natural condition.

Where, PH = Plant height, D50% F= days to 50% flowering, DM= days to maturity, GFD= grain filling duration, SLWOA= spike length without awns, SLWA = spike length with awns, SPP = spikes per plant, GY= grain yield, TGW= thousand grain weight, AUDPC= Area under disease progress curve.

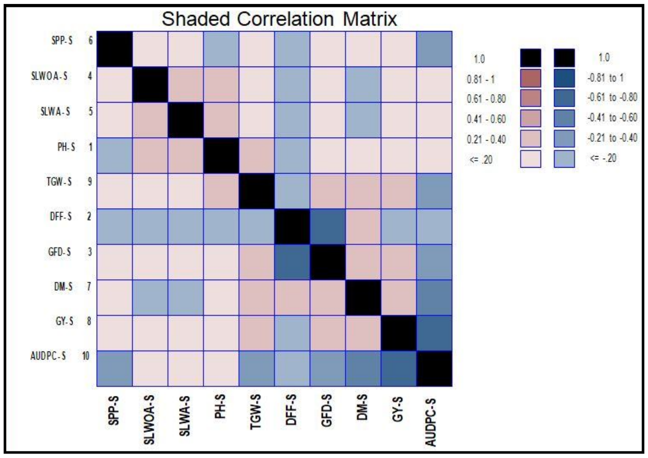

Fig. 2: Degree of association analysis matrix under biotic stress.

Where, PH = Plant height, D50% F= days to 50% flowering, DM= days to maturity, GFD= grain filling duration, SLWOA= spike length without awns, SLWA = spike length with awns, SPP = spikes per plant, GY= grain yield, TGW= thousand grain weight, AUDPC= Area under disease progress curve.

investigation, R^2 and the residual effect observed for path analysis is 0.533 and 0.683 respectively. The residual effect indicates that the component characters under study were responsible for about 32% of the variability in AUDPC.

Grain yield exerted considerable negative indirect effects on AUDPC via days to maturity and 1000 grain weight while positive indirect effect via grain filling duration. Days to maturity had a negative indirect effect on AUDPC via grain yield while positive indirect effect via grain filling duration and days to 50% flowering. Grain filling duration as most important yield contributing traits which merit due consideration at the time of devising selection strategy aimed at developing high yielding varieties in barley.

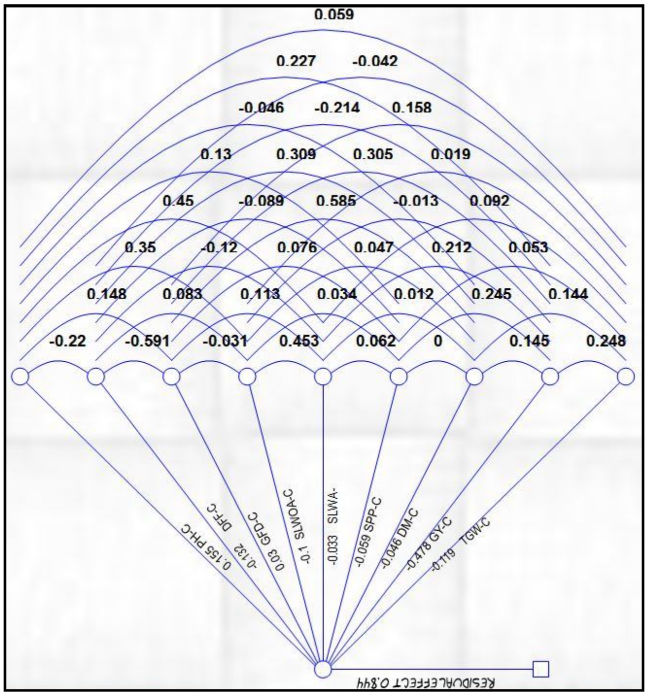

Fig. 3: Cause and effect analysis diagram under natural condition.

Where, PH = Plant height, D50% F= days to 50% flowering, DM= days to maturity, GFD= grain filling duration, SLWOA= spike length without awns, SLWA = spike length with awns, SPP = spikes per plant, GY= grain yield, TGW= thousand grain weight, AUDPC= Area under disease progress curve.

Negative correlation of AUDPC with seed yield per plant was mainly due to its direct negative effect on seed yield indicated that the negative effect of spot blotch disease on seed yield per plant.

Since resistance is the main aim, it is, therefore, interesting to note that characters showing negative and direct association with AUDPC may be quite useful for the formation of selection criteria for the breeding of spot blotch resistant varieties, but characters showing the positive and direct effect with spot blotch AUDPC are yield contributing traits.

It is, therefore, suggested that by sacrificing such important yield components the breeder has to put more emphasis on other yield contributing characters in such a way so that yield is not sacrificed for resistance. Therefore, the characters which show negative direct effect and highly significant negative association with spot blotch resistance may play a major important role in the

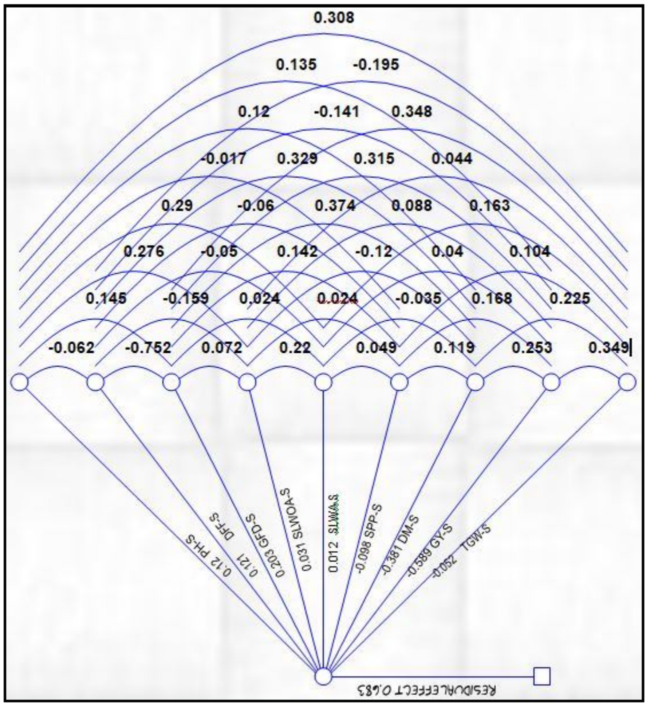

Fig. 4: Cause and effect analysis diagram under biotic stress condition.

Where, PH = Plant height, D50% F= days to 50% flowering, DM= days to maturity, GFD= grain filling duration, SLWOA= spike length without awns, SLWA = spike length with awns, SPP = spikes per plant, GY= grain yield, TGW= thousand grain weight, AUDPC= Area under disease progress curve.

formation of indirect selection criteria for breeding the spot blotch resistant genotypes.

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Abbreviation:- PH = Plant height, D50% F= days to 50% flowering, DM= days to maturity, GFD= grain filling duration, SLWOA= spike length without awns, SLWA = spike length with awns, SPP = spikes per plant, GY= grain yield, TGW= thousand grain weight, AUDPC= Area under disease progress curve.

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