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Selection of superior transgressive segregants from NBS-5 strain of white button mushroom

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ABSTRACT

Present study is an attempt for the genetic improvement of commercially important white button mushroom through quantitative genetics. Systematic and continuous breeding programme is the critical for enhancing crop productivity. Among the cultivated edible mushrooms, white button mushroom occupies fourth prominent place in terms of production across at the global level. Single spore isolates of high yielding non browning hybrid strain NBS-5 was selected for the present study. A total number of 189 single spore isolates were isolated from the strain and evaluated under controlled environment conditions for the selection of segregants in the F_2 generation. Analysis of variance indicated significant differences among all the genotypes for yield and yield related traits. Genetic analysis showed significant influence of the growing environment on the expression of different traits. High heritability coupled with high genetic advance was observed for the average fruit body weight and stipe length suggesting that yield can be improved through direct phenotypic selection.

Key words: Cluster analysis, correlation, euclidean distance, variability

Agaricus bisporus (Lange) Imbach popularly known as the white button mushroom is ranked fourth (15%) amongst most cultivated edible mushroom in the world (Royse *et al.,* 2017) next to shiitake (22%), oyster (19%) and black ear mushroom (17%). With the innovation and flow of technologies, mushroom farming has expanded rapidly both in terms of production and productivity (Dhar, 2014). The global mushroom production has increased from a mere 0.5 million tonnes (MT) in 1961 to 34.8 MT in 2013 (Royse *et al.,* 2017, Singh *et al.,* 2017). Though the cultivation of this mushroom was prominent in the western hemisphere in early phases of its growth (Atkins, 1974) but it is steadily making inroads into the eastern part of the globe (Sonnenberg, 2011) in the recent past. Presently, China is the leading producer of white button mushroom contributing around 54% (2.37 million tons) of the total world production (Royse *et al.,* 2017). White button mushroom is the most cultivated mushroom in India contributing nearly 72% to the total Indian mushroom production (Sharma *et al.,* 2017). The productivity of this mushroom has been increased considerably in the last few years due to the improvements in the cultivation technologies. Despite the economic significance, the systematic breeding efforts for genetic improvement of this species is limited both at global and Indian level. Even the white strain in *A. bisporus* was due to a spontaneous mutation in 1926 in the beds of Low Downing of Downing town, PA

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and Lambert propagated it by means of multispore culture for selection a pure 'white strain' (Lambert, 1960). The commercial cultivars available to the Indian growers are low yielders due to their poor adaptability to the fluctuating growing conditions (Yadav, 2003).

Breeding in white button mushroom is difficult because of its secondary homothallic sexual behavior. However, better understanding of the life cycle of this species enabled the application of various breeding tools for genetic improvement. The cross breeding work on button mushroom started in 1970s at Mushroom Experimental Station in Horst, The Netherlands. The first hybrid strain developed was U_1 and later its seggregant single spore isolate U_3 got wide acceptance in the mushroom world with higher yields and better quality (Fritsche, 1986). Further genetic improvement work in this species was stalled and the new varieties released eventually are sharing close genetic background with these strains (Gao *et al.,* 2011). Breeding programme in India started in 1984 by Bhandal and Mehta and released the first hybrids NCH 102 in 1997. Later the high yielding hybrid strain NBS-5 released from Indian Council of Agricultural Research (ICAR)-Directorate of Mushroom Research (DMR), Solan overcame the problem of browning and became a prominent hybrid strain (Kamal *et al.,* 2019). Considering the limited genetic base of the *Agaricus bisporus*, the genetic improvement strategy should involve the hybridization of genotypes with desirable traits and further selection of superior lines from the F_2 population where transgressive segregation takes place. The information on the heritability of economically important traits plays critical role in the quantitative genetics. The success of selection largely depends on the fixation of segregants in the early generations. It is being hypothesized that frequency of transgressive segregants is positively correlated with the genetic distance between the parental lines which also helps in prediction of heterotic crosses (Rieseberg *et al.,* 1999). The present study aimed to identify the transgressive segragants with high yielding potential from the non browning hybrid strain NBS-5.

MATERIALS AND METHODS

The experiment was conducted in the environmentally controlled cropping rooms of ICAR-DMR, Solan in the year 2018-19.

Isolation of single spore isolates (SSIs): Matured and healthy fruiting bodies of non browning hybrid strain NBS-5 were selected and the spore prints were collected under aseptic conditions using pre-sterilized petri dishes. The spore mass then stored at 4°C until further use. Single spores were isolated using serial dilution technique on the standardized media containing dextrose – 5.0 g; MgSO4 –0.25 g; KH2PO4 – 0.95 g; succinic acid – 1.25 g; Agar –15.0 g; water 500 mL and pH –5.0. Placing *Agaricus* mycelium on the lid triggered the spore germination (Singh and Kamal, 2011). A total of 300 SSIs of non browning hybrid strain NBS-5 was isolated and cultured on the PDA culture medium. Out of which, 206 SSIs were progressed for spawn development on the wheat grains.

Yield evaluation trials: The grain spawn obtained from these 206 SSIs was inoculated in pasteurized compost prepared by the short method of compositing (Sinden and Hauser, 1951). The yield trails were conducted following the standard package of practices for raising white button mushroom crop. The yield trails were conducted in complete randomized block design (CRBD) with three replications. The quantitative data was recorded in respect of no. of days to first flush, pileus length (cm), pileuswidth (cm), stipe length (cm), stipe width (cm), fruit body weight (g) and yield (g).

Statistical analysis: Analysis of variance (ANOVA) for different characters was carried out as suggested by Panse and Sukhatme (1969). The data was estimated for variability parameters such as heritability, genetic advance, phenotypic and genotypic coefficient of variance. The genotypic, phenotypic and environmental correlations were studied between each character and yield. The path correlation was also estimated to identify traits for selection of high yielding stains using OPSTAT software (Sheoran *et al.*, 1998). The data was subjected to analysis genetic variability and Principal Component Analysis using XLSTAT software (2017). The genetic variability and cluster analysis were studied using Euclidean distance matrix (Hoque and Rahman, 2007).

RESULTS AND DISCUSSION

The phenotypic data obtained in the study was subjected to Analysis of Variance (ANOVA) for number of days taken for first flush, average fruit body weight, total yield and yield attributing factors such as pileus and stipe length, pileus and stipe width in the F_2 population and their parental line and is presented in table 1. According to the ANOVA, highly significant differences among the genotypes were observed for the characters studied. All of the characters showed highly significant differences except number of days to first flush, which shows that the single spore isolates showed variation in respect of all the characters.

Genetic variability

Genetic parameters such as phenotypic and genotypic coefficient of variability (PCV, GCV), heritability (h^2), genetic advance (GA) and genetic advance as percent of mean (GAM) and mean performance for the seven yield and yield related traits are presented in Table 2.

Estimation of genetic parameters like phenotypic and genotypic coefficients of variation, heritability and genetic advance helps in portioning of total variability

Table 1. ANOVAfor quantitative traits of 189 single spore isolates along with control

Source of variation	df	No. of days to first flush	Pileus length	Pileus Width	Stipe length	Stipe width	Fruit body weight	Yield
Rep	2	9.83	49.36	4.89	8.69	2.16	9038.85	414603.1
Strains	190	1.26*	0.33**	0.18**	0.46**	0.12**	51.54**	49454.48**
Error	380	0.97	0.08	0.09	0.10	0.09	15.70	36653.38

* Significant at 0.05 probability value; ** significant at 0.01 probability value

Table 2. Genetic variability parameters for 190 SSIs of NBS-5 hybrid of button mushroom

	No. of days to first flush	Pileus length	Pileus Width	Stipe length	Stipe width	Fruit body weight	Yield
PCV	2.96	10.24	16.87	27.51	11.64	26.43	40.07
GCV	0.90	7.21	8.91	20.40	4.26	17.37	12.94
ECV	2.82	7.27	14.33	18.44	10.83	19.92	37.92
h2	9.17	49.54	27.88	55.03	13.37	43.22	10.43
GA	0.20	0.42	0.20	0.53	0.09	4.68	43.45
GAM	0.56	10.45	9.69	31.18	3.21	23.53	8.61
Mean	34.91	4.00	2.06	1.72	2.71	19.90	504.88
S.E.	0.57	0.17	0.17	0.18	0.17	2.29	110.54
C.D.(5%)	1.57	0.47	0.47	0.51	0.47	6.34	306.39
CV(%)	2.82	7.27	14.33	18.45	10.83	19.92	37.92

present in the genetic stock into heritable and non-heritable components. Comparing the PCV and GCV values also provides an insight into the influence of environment on the desirable traits. Higher PCV values observed in the present study for all the seven characters than the GCV values suggested significant influence of growing conditions on the expression of these traits. Hence selection of strains in *A. bisporus* exclusively based on the phenotypic characters may be inadequate. However, fruit body weight and stipe length showed maximum PCV and GCV values with relatively high genetic advance. The least PCV (2.96) and GCV (0.90) values were recorded for the trait number of days taken for first flush. The low PCV and GCV values of time taken to first flush show limited scope of selection / genetic improvement using this trait. Moderate level of PCV and GCV values were observed with pileus length, pileus width and stipe width which indicates the presence of moderate genetic variability in these characters.

The mean performance of SSIs for the seven characters taken for the study indicated that significant variability present among the segregants. The data revealed the existence of adequate variability in the genetic material to carry out further analysis. The days to first flush, an indication of early crop, was found with a mean value of 34.91 days from the day of spawning. Mean average fruit body weight was recorded 19.90g. Pileus length and width which attributes to the fruit body quality were recorded with a mean value of 4.00 cm and 2.06 cm, respectively. The stipe length and width were observed with a mean value of 1.72 cm and 2.71cm, respectively. The estimation of heritability (h^2) helps in determination of proportion of heritable variation out of the total variability. These values can be more effectively used with greater accuracy when heritability is studied in concurrence with genetic advance values (Johnson *et al.* 1955). The analysis showed that the maximum broad sense heritability was observed for stipe length (55.03%), pileus length (49.54%) and average fruit body weight (43.22%). Further, the genetic advance of the average fruit body weight was found highest (4.68) followed by stipe length (0.53) and pileus length (0.42). High heritability coupled with high genetic advance is considered to be the most heritable trait during selection of the strains. Thus average fruit body weight was found to be the most heritable trait in case of white button mushroom. High h^2 and genetic advance observed in the characters like stipe length and average fruit body weight clearly indicating that these traits are expressing additive gene action and enables the breeder to exercise the phenotypic selection for genetic improvement. High heritability coupled with moderate genetic advance observed for pileus length and pileus width shows the significant influence of growing environment on their expression and also exhibits the non-additive gene action for these traits.

Clustering and PCA analysis

The data obtained was analysed for genetic variability, cluster analysis and Principal Component Analysis. Clustering based Euclidean distance showed formation three distinct clusters (Fig 1). Cluster 1 contained 56, cluster 2 contained 99 and cluster 3 contained 35 genotypes. Cluster 1 and 2 showed an elucidian distance of 166.51 while cluster 1 and 3 showed 338.09 and cluster 2 and 3 showed 177.67. Dendrogram produced by the eluciadian distance showed high degree of dissimilarity amongst cluster and also the analysis showed that the phenotypic characters of different clusters have significant differences. It could also be observed that cluster 3 with 35 genotypes showed maximum yield along with lowest time for first harvest. Five SSIs namely NBS5-2207, NBS5-2206, NBS5-2024, NBS5-2129 and NBS5-2171 having maximum mean yield are clustered in cluster-3.

Principal component analysis of data divided the total variation in to two principal axis (PC1 & PC2) showing 58.35% variation. It also showed that different characters of 189 strains of white button mushroom are highly correlated, negatively correlated or not correlated. The analysis showed that yield of

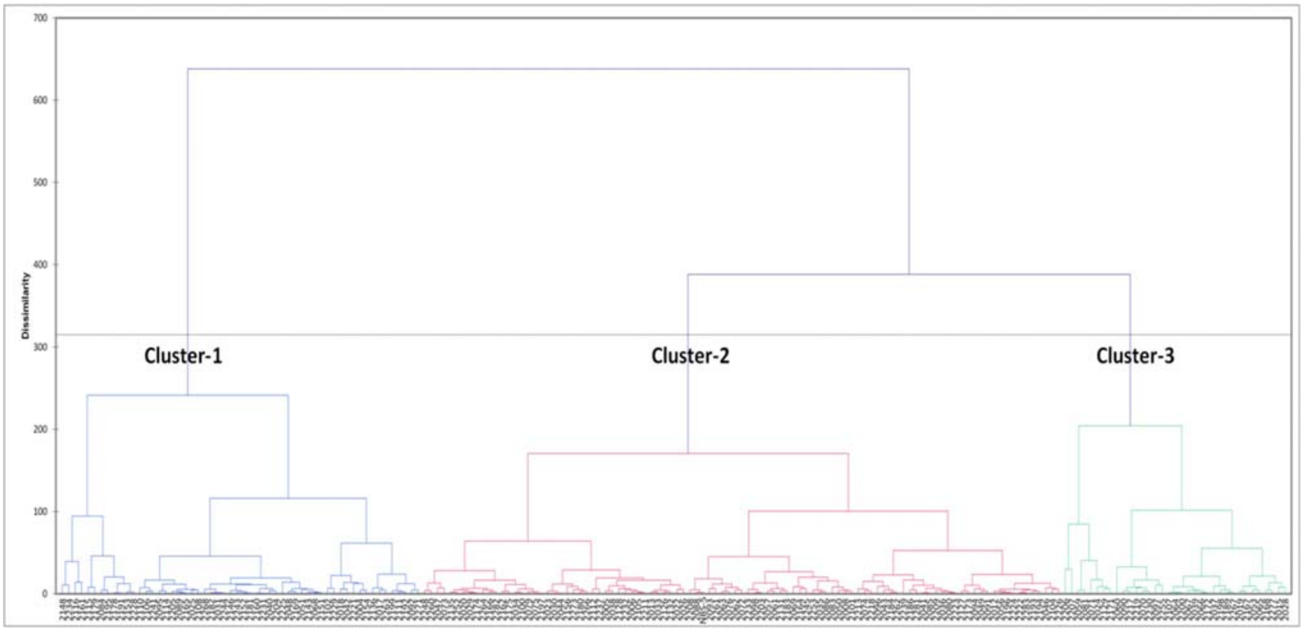


Fig. 1. Clustering analysis of 189 SSIs of NBS-5 hybrid along with control showing three clusters

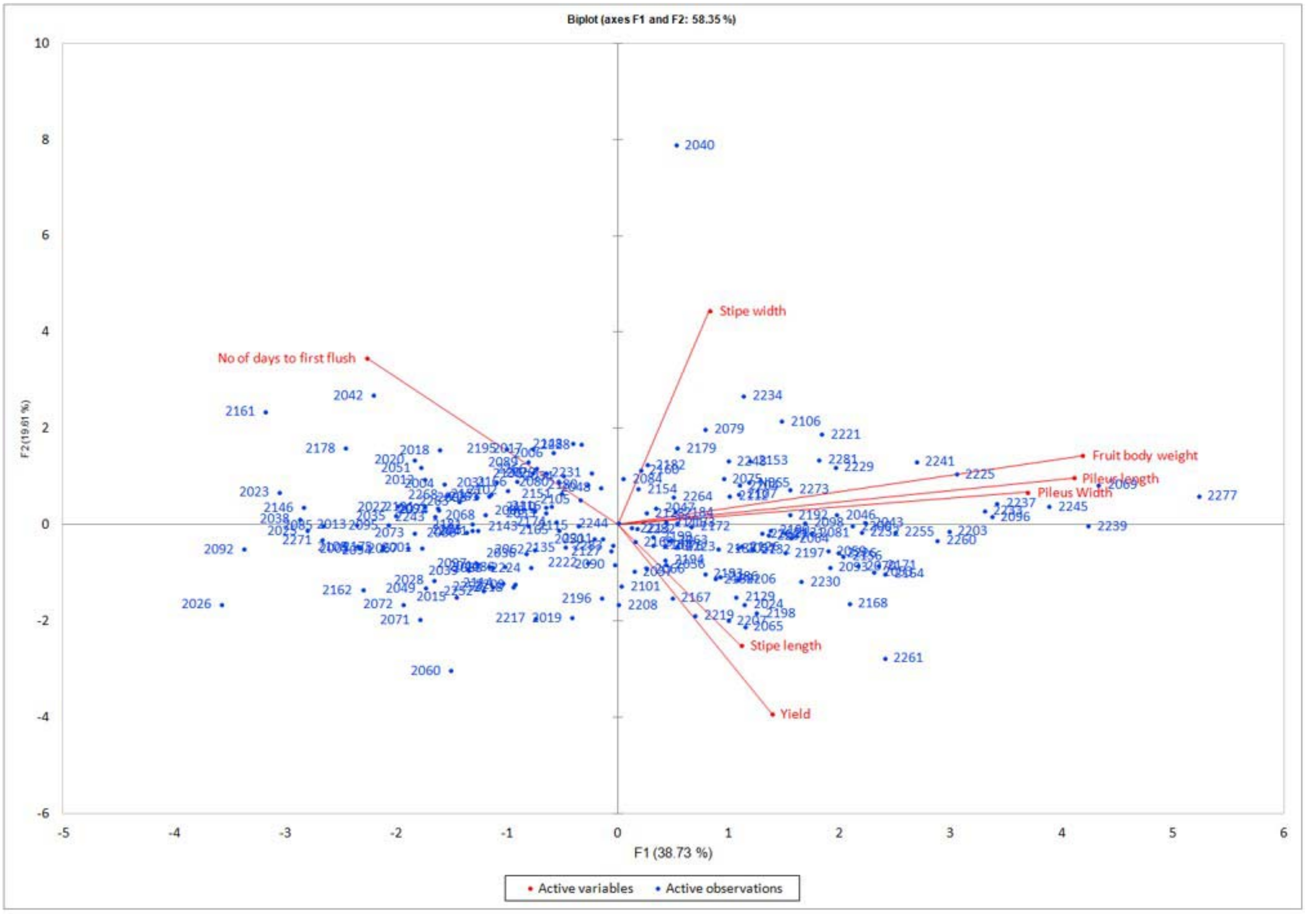
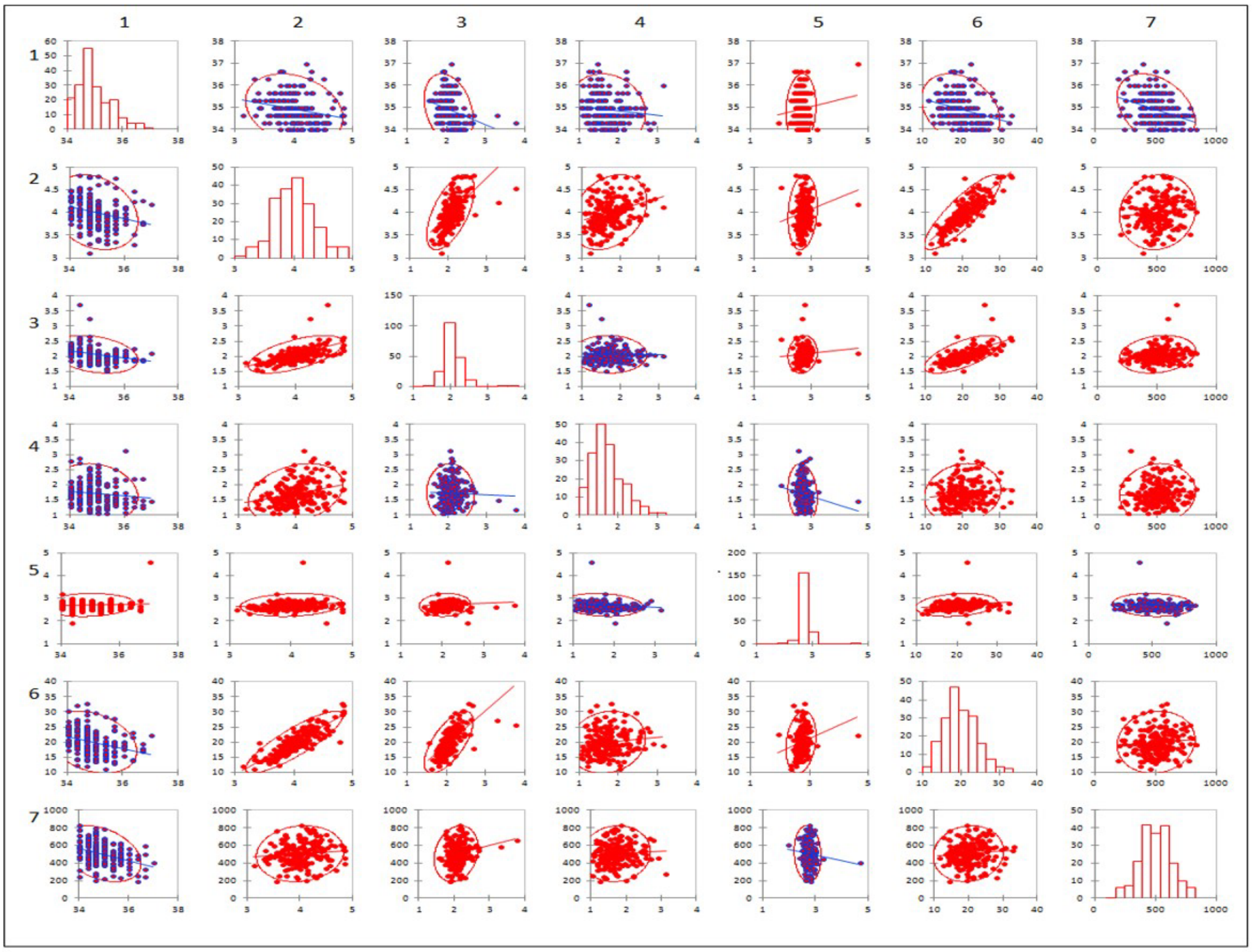


Fig 2. PCA-biplot for 189 SSIs of NBS-5 of button mushroom along with control i.e NBS-5

all the test isolates was found highly correlated with stipe length, moderately correlated with fruit body weight, pileus length and pileus width. The negative correlation of yield was observed with number of days to first flush (Fig. 2).

The data on different characters of 189 test isolates of button mushroom was subjected to scatter plot analysis using XLSTAT software. The results obtained are presented in Fig 3. The scatter plot was drawn using the correlation between different characters . The analysis segregated the out performers based on the correlation of two characters taken in to consideration at one point of time. The correlation between days taken to first harvest and yield showed that the isolates taking less time to first harvest are generally the higher yielders and out of 191 isolates 9 isolates have shown deviation from the prediction eclipse. The analysis also showed that the yield of the isolate is increasing with the average fruit body weight, pileus length and pileus width and very less number of isolates are violating this assumption by deviating from the prediction eclipse. This analysis also suggested that these three characters *i.e.* average fruit body weight, pileus length and pileus width can be used for the selection of high yielding isolates in white button mushroom.



1- No of days to first flush; 2-Pileus length; 3-Pileus Width; 4-Stipe length, 5-Stipe width; 6-Fruit body weight; 7-Yield

Fig. 3. Scatter plot for 191 SSIs of NBS-5 of button mushroom showing prediction eclipse and isolates deviating from the eclipse

Correlation and path correlation

The genotypic, phenotypic, environmental correlation and path correlation was also studied in SSIs of the NBS-5 hybrid. For yield, strong positive genotypic correlation was observed from pileus width followed by fruit body weight and pileus length while a strong negative genotypic correlation was observed for number of days to first flush and stipe width. The fruit body weight showed highest positive correlation with pileus width followed by pileus length which indicates that the increase in fruit body weight in button mushroom is due to increase in pileus shape (length and width) (Table 3). To understand the above phenomenon, the genotypic path correlation was studied. The path analysis showed that the pileus width

Table 3. Genotypic (Gxy), Phenotypic (Pxy) and Environmental (Exy) correlation between seven quantitative traits in button mushroom

G_{XY},P_{XY},E_{XY}	Pileus length	Pileus Width	Stipe length	Stipe width	Fruit body weight	Yield
No. of days to first flush	- 0.68** -0.09* 0.07	-0.80** -0.14** -0.01	-0.34** -0.04 0.05	0.68** -0.03 -0.11**	-0.84** -0.10* 0.09*	-0.39** -0.35** -0.34**
Pileus length		0.83** 0.45** 0.24**	0.32** 0.26** 0.18**	0.18** 0.16** 0.18**	0.91** 0.82** 0.75**	0.22** 0.06 0.02
Pileus Width			-0.05 -0.01 0.02	0.23** 0.04 0.00	0.96** 0.48** 0.23**	0.46** 0.12** 0.05
Stipe length				-0.30** -0.09* -0.01	0.11** 0.16** 0.20**	0.15** 0.04 0.00
Stipe width					0.26** 0.21** 0.22**	-0.37** -0.03 0.01
Fruit body weight						0.23** 0.05 0.00

Table 4. Genotypic path correlation between seven quantitative traits in button mushroom

Effects		Indirect effect on yield						
Characters	Direct effect on Yield	No. of days to first flush	Pileus length	Pileus Width	Stipe length	Stipe width	Fruit body weight	Genotypic Correlation Yield
No. of days to first flush	0.09	0.00	0.09	-4.75	-0.39	0.01	4.56	-0.39
Pileus length	-0.13	-0.06	0.00	4.95	0.37	0.00	-4.91	0.22
Pileus Width	5.93	-0.07	-0.11	0.00	-0.06	0.00	-5.23	0.46
Stipe length	1.15	-0.03	-0.04	-0.31	0.00	0.00	-0.61	0.15
Stipe width	0.01	0.06	-0.02	1.34	-0.35	0.00	-1.41	-0.37
Fruit body weight	-5.42	-0.08	-0.12	5.71	0.13	0.00	0.00	0.23
Residual	**- 0.62**							

have direct effect on the yield while pileus length have indirectly effect on yield through path of pileus width. Moreover, fruit body weight also has indirect effect on yield through pileus width. Thus, it can be concluded that the pileus width is the most important criteria for indirect yield selection in button mushroom.

CONCLUSION

Fungal breeding is comparatively a new science. The various principal of plant and animal breeding stand true in the fungus too. The present study showed the identification of transgressive fertile SSIs form F_2 population of NBS-5 non browning hybrid of button mushroom. In the study based on cluster analysis five transgressive segregants / SSIs were selected namely NBS5-2207, NBS5-2206, NBS5-2024, NBS5-2129, and NBS5-2171 which also showed high yield too. The study also showed significance of Euclidian distance based cluster analysis for selection of the promising SSIs/ strains in button mushroom. The selection criteria for button mushroom breeding was figured in study and found that average fruit body weight possessed high heritability with high genetic advance which can be used for selection of high yielder stains. During selection, it is to be also taken into consideration that average fruit body weight is indirectly influenced by pileus width thus both traits are responsible for high yield. In light of our study, it can be concluded that pileus width cannot be directly taken as sole criteria for selection due to its low heritability.

REFERENCES

1. Atkins, F.C. 1974. *Guide to Mushroom Growing*. London : Faber, 122p

2. Dhar, B.L. 2014. Changing global scenario in mushroom industry. In: M. Singh 342 (Ed.) *8th International conference on mushroom biology and mushroom products* (pp. 602-605). ICAR, Directorate of Mushroom Research, New Delhi, India.

3. Gao, W., J. Baars, A.S.M. Sonnenberg and R. Visser. 2011. Inheritance pattern of bruising sensitivity trait in *Agaricus bisporus*. In: Mushroom Biology and Mushroom Products. *Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products*, Arcachon, France, 4-7 October, 2011. Volume 1. Savoie J, M. Foulongne-Oriol, M. Largeteau, G. Barroso (eds). pp 43–51, Institut National de la Recherche Agronomique (INRA).

4. Hoque, M.N. and L. Rahman. 2007. Estimation of euclidean distance for different morpho-physiological characters in some wild and cultivated rice genotypes (*Oryza sativa* L.). *Journal of Biological Sciences* **7(1):** 86-88. https://doi.org/10.3923/jbs.2007.86.88

5. J.W. Sinden and E. Hauser. 1951. The Short Method of Composting. *Mushroom Sci* **1(1):** 52-58.

6. Johnson, H.W., H.F. Robinson and R.E. Comstock. 1955. Estimates of genetic and environmental variability in soybean. *Agronomy Journal* **47:** 314-318.

7. Kamal, S., V.P. Sharma, M. Gupta, A. Barh and M. Singh. 2019. Development, evaluation and characterization of browning-resistant hybrids of white button mushroom (*Agaricus bisporus*). *Indian J Genet* **79(1):** 131-140. DOI: 10.31742/IJGPB.79S.1.2

8. Lambert, E. 1960. Improving spawn cultures of cultivated mushrooms. *Mushroom Science* **4:** 33–51.

9. Manjit, S. and S. Kamal. 2011. Conventional and molecular approaches for breeding button mushroom. *Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products*

(ICMBMP7) at Arcachon, France 4-7 Oct 2011. pp 35-42.

10. Rieseberg, L., M. Archer and R. Waynw. 1999. Transgressive segregation, adaptation and speciation. *Heredity* **83:** 363-372.

11. Royse, D.J., J. Baars and Q. Tan. 2017. Current Overview of Mushroom Production in the World. In: *Edible and Medicinal Mushrooms* (eds C. Z. Diego and A. Pardo Giménez). doi:10.1002/9781119149446.ch2.

12. Sharma, V.P., S.K. Annepu, Y. Gautam, M. Singh and S. Kamal. 2017. Status of mushroom production in India. *Mushroom Res* **26(2):** 111-120.

13. Sheoran, O.P., D.S. Tonk, L.S. Kaushik, R.C. Hasija and R.S. Pannu. 1998. Statistical Software Package for Agricultural Research Workers. Recent Advances in information theory, Statistics & Computer Applications by D.S. Hooda& R.C. Hasija Department of Mathematics Statistics, CCS HAU, Hisar (139-143).

14. Singh, M., S. Kamal and V.P. Sharma. 2017. Status and trends in world mushroom production-I. *Mushroom Res* **26(1):** 1-20.

15. Sonnenberg, A.S.M., J.P. Baars, P.M. Hendrickx, B. Lavrijssen, W. Gao, A. Weijn and J. Mes. 2011. Breeding and strain protection in the button mushroom *Agaricus bisporus*. In: *Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products (ICMBMP7)* pp 7-15.

16. XLSTAT 2017. Data Analysis and Statistical Solution for Microsoft Excel. Addinsoft, Paris, France (2017).

17. Yadav, M.C. 2003. Molecular breeding for development of genetically improved strains and hybrids of *Agaricus bisporus*. In: *Current vistas in mushroom biology and production*. Pp. 261-274.