Investigation on the influence of seedling's physiological attributes on productivity in black pepper

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2 authors:

Krishnamurthy Ks
Indian Institute of Spices Research

Chempakam Bhageerathy
Indian Institute of Spices Research

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Investigation on the influence of seedling's physiological attributes on productivity in black pepper

K.S. Krishnamurthy and B. Chempakam
Indian Institute of Spices Research, Marikunnu P.O., Calicut 673 012

ABSTRACT

Black pepper is a perennial crop which takes a minimum of five years for yield stabilization. Hence, any seedling character which reflects its productivity may be of use in identifying the probable high yielding types at an early stage. Present study this to analyse the influence of some physiological and biochemical parameters such as leaf and stem carbohydrate status, nitrate reductase and sucrose phosphate synthase activities and leaf gas exchange parameters during pre-bearing and bearing stages on productivity in black pepper. Field grown plants (1-2 years) were used for the study. Results revealed that leaf starch and sucrose phosphate synthase activity did not show significant correlation with productivity. Leaf photosynthesis, nitrate reductase activity and stomatal conductance showed significant positive correlation while leaf temperature had significant negative correlation with productivity. It was concluded that in black pepper, physiological traits such as high nitrate reductase activity, photosynthetic rate and stomatal conductance and low leaf temperature may be useful in identifying high yielding types during juvenile stage itself.

Key words: Black pepper, starch, nitrate reductase, sucrose phosphate synthase, photosynthetic rate, stomatal conductance, leaf temperature, transpiration rate.

INTRODUCTION

Crop productivity is a composite trait controlled by genetic factors but can be modified by the environment to reach its genetic potential. In general, the yield of a variety will be below its genetic potential due to unfavourable environmental conditions or diseases or due to poor management. Equally important is the plant's physiological and biochemical functions. Impairment of plant metabolic functions definitely hampers productivity. Both growth and yield attributing factors control productivity. Developmental/morphological traits have a strong relationship with productivity in some crops. Identification of physiological/biochemical determinants linked to growth that can be quantified early in seedling development may be important for breeding purposes. Carbohydrate metabolism is a good candidate for such an approach. But the question remains to be answered is that are there any physiological/biochemical traits of a crop which reflect its productivity during seedling stage itself? Nitrate reductase (NR) and sucrose phosphate synthase activities are reported to have some relationship with crop yields in a few species. Activity of NR is linked to productivity in wheat (Blackwood and Hallam, 1), tea (Venkatesan and Ganapathy, 20; Wickremasinghe *et al*., 21) etc. Laporte *et al*. (10) reported that one of the transgenic plants overexpressing SPS had higher yield than control. Photosynthetic and gas exchange parameters have also shown relationship with productivity in some species (Tafur *et al.*, 17; El Sharkawy *et al*., 6). Accumulation of carbohydrates and their metabolism is also linked to productivity. It is interesting to see if such relationship exists in black pepper also which helps to eliminate low yielders quickly without wasting much time, money, energy and space for germplasm evaluation. Hence, the objectives of the present study were to evaluate contrasting genotypes of black pepper (high and low yielders) for nitrate reductase and sucrose phosphate synthase activities, starch accumulation, and photosynthesis and gas exchange parameters during seedling stage to investigate if any of these parameters influence productivity.

MATERIALS AND METHODS

High and low yielding black pepper accessions were collected from experimental farm, Indian Institute of Spices Research, Peruvannamuzhi. Accessions which recorded at least 4 kg fresh berries per vine for at least 3 years were selected as high yielders (Panniyur-1, OPKM, HP813, HP 780, HP 1411, Accession number (Accn) 1619, 1481, 1041 and 4129) and those which recorded less than 2 kg fresh per vine were selected as low yielders (Accn 1157, 840, 1120, 4132, 4095, 4112, 1607, 1535 and 1467). Youngest fully matured leaves ($4^{th}/5^{th}$ leaf from the top) were used for all assays. Field

Corresponding author's E-mail: kskrishnamurthy@gmail.com

grown plants (1-3 years old) were used for the study. Plants were watered regularly and all the plant protection measures were followed as per the package of practices standardized for black pepper. Starch content, nitrate reductase (NR) and sucrose phosphate synthase (SPS) activities were monitored both during pre-bearing and bearing periods. Pre-bearing period refers to the period before spike initiation (April-May). Bearing period refers to the middle of berry development period (September-October). Photosynthesis and gas exchange parameters were measured using LCA 4 leaf microclimate system supplied by ADC, UK. Though there is not much diurnal variation in photosynthetic rate in black pepper, gas exchange measurements were made between 9.30 and 11.30 AM during October to December.

Starch was analysed using anthrone method (Hodge and Hofreiter, 7). Nitrate reductase activity was assayed *in-vivo* as per Sadasivam and Manickam (14). Sucrose phosphate synthase activity was assayed as per Dochlert and Huber (4) with suitable modifications. SPS was extracted in 50 mM HEPES NaOH, pH 7.5 containing 5 mM $MgCl_2$, 1 mM EDTA, 2.5 mM DTT, 10g/l BSA, 2 % PVP, 0.5 ml/l tween 20 and 1 mM PMSF. The homogenate was centrifuged at 13,000 g for 20 minutes and the supernatant was collected and assayed for SPS activity. Assays were initiated by addition of 100µl extract to a reaction mixture containing 50mM HEPES NaOH, pH 7.4, 15 mM $MgCl_2$, 5 mM UDPG, 1 mM EDTA, 5 mM fructose 6 phosphate and 25 mM glucose 6 phosphate. Blanks lacking fructose 6 phosphate and glucose 6 phosphate were included for each sample. Assays were terminated by the addition of 1M NaOH. The fructose was estimated using resorcinol thiourea reagent as per Roe and Papadopoulos (13). The SPS activity was expressed as µ moles of fructose g^{-1} fresh weight. The results were statistically analysed using SPSS package and various tests such as ANOVA, t-test and bivariate correlations were employed to analyse the data.

RESULTS AND DISCUSSION

Table 1 shows the starch content of high and low yielding black pepper accessions in various parts during bearing period. Significant differences were noticed in leaf, stem and berry starch content among the accessions. The leaf starch content of selected accessions ranged between 6.35 to 7.52 %. The mean leaf starch content of high (7.0 %) and low yielders (6.81 %) were on par. HP 1411 showed highest leaf starch content of 7.52 % while accn 1481 showed the least (6.48 %). The stem starch content ranged from 6.15 to 7.20 %. The mean stem starch content of high (6.71 %) and low yielders (6.70 %) were on par. OPKM recorded highest starch in stem (7.2 %) followed by HP 1411 (7.10) while accn 1481 showed the least (6.17 %). The mean berry starch content ranged between 13.12 to 15.24 %. It was significantly higher in high yielders (14.69 %) than that of low yielders (13.66 %). The highest berry starch content (15.24 %) was recorded in HP 780 and the lowest (13.12 %) in accn 1467. The results indicated that high yielders comparatively accumulated more starch during the berry development period.

Among the gas exchange parameters (Table 2), the photosynthetic rate ranged from 3.87 to 1.98 µ moles m^{-2} s^{-1}. The mean photosynthetic rate of high yielders was 3.1 µ moles m^{-2} s^{-1} which was significantly higher (t test, p = 0.03) than that of low yielders (2.66 µ moles m^{-2} s^{-1}). The highest photosynthetic rate was observed in Panniyur -1 (3.87 µ moles m^{-2} s^{-1}) while accn 4112 showed lowest photosynthetic rate (1.98 µ moles m^{-2} s-

Table 1. Starch content (%) of black pepper accessions with varying yielding levels.

High yielder (Accn/variety)	Leaves	Stem	Berries	Low yielder (Accn)	Leaves	Stem	Berries
Panniyur-1	7.26	6.95	14.85	1157	7.04	6.86	13.76
1041	6.97	6.81	13.92	840	6.97	6.71	13.20
OPKM	7.14	7.20	15.21	1120	6.84	6.24	13.35
HP813	6.52	6.76	13.74	4132	7.25	7.02	14.42
HP780	6.86	6.54	15.24	4095	6.47	6.15	14.21
HP1411	7.52	7.10	14.76	4112	6.52	6.84	13.83
1619	7.02	6.38	14.91	1607	7.16	6.97	13.47
1481	6.48	6.17	14.62	1535	6.35	6.76	13.54
4129	7.21	6.45	14.93	1467	6.66	6.71	13.12
Mean	7.00	6.71	14.69	Mean	6.81	6.70	13.66
CD (0.05)	0.45	0.61	0.74		0.45	0.61	0.74

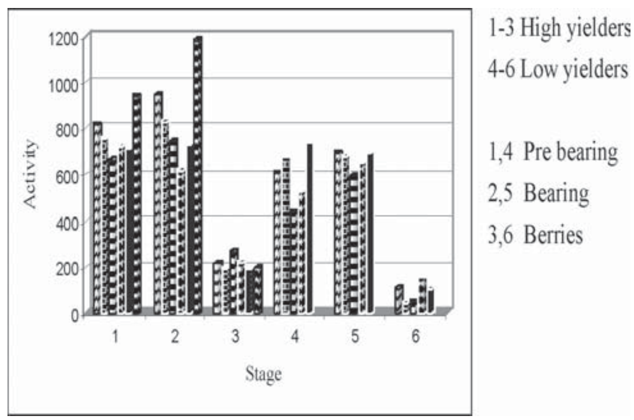
Table 2. Gas exchange parameters of black pepper accessions with high and low productivity levels.

High yielder (Accn/variety)	A	E	gs	T_L	Low yielder (Accn)	A	E	gs	T_L
Panniyur-1	3.87	1.61	0.08	32.6	1157	2.96	1.42	0.05	33.7
1041	3.25	1.35	0.06	33.3	840	3.15	1.51	0.06	32.9
OPKM	3.41	1.41	0.06	33.4	1120	2.47	1.19	0.04	33.8
HP813	2.94	1.32	0.04	33.2	4132	3.21	1.42	0.05	33.7
HP780	3.03	1.34	0.05	32.9	4095	2.71	1.05	0.04	33.8
HP1411	2.79	1.14	0.06	33.7	4112	1.98	1.01	0.04	33.7
1481	2.26	1.05	0.04	33.1	1607	2.64	1.26	0.04	33.6
4129	3.14	1.52	0.06	33.0	1535	2.17	1.17	0.05	33.4
Mean	3.10	1.34	0.056	33.2	Mean	2.66	1.25	0.046	33.6
CD (0.05)	0.32	NS	NS	NS		0.32	NS	NS	NS

A- Photosynthetic rate (ì moles m^{-2} s^{-1}) g_s – Stomatal conductance.
E- Transpiration rate (m moles) T_L- Leaf temperature (°C).

^{1}). The transpiration rate ranged from 1.05 to 1.61 m moles. The mean transpiration rate of high and low yielders was 1.34 and 1.25 m moles respectively and were on par. Panniyur-1 showed highest transpiration rate of 1.61 m moles while accn 4112 showed the least transpiration rate of 1.01 m moles. Stomatal conductance ranged from 0.04 to 0.08. The mean stomatal conductance of high yielders was 0.056 which was significantly higher (t test, p = 0.03) than that of low yielders (0.046). Highest stomatal conductance of 0.08 was recorded in Panniyur-1. The leaf temperature ranged from 32.6 to 33.8 °C. The mean leaf temperature of high yielders (33.2°C) was significantly lower (t test, p = 0.02) than that of low yielders (33.6°C). Lowest leaf temperature of 32.6°C was recorded in Panniyur-1.

Nitrate reductase activity was higher in leaves compared to the berries in all the accessions (Fig.1) irrespective of their yield levels. Further, in leaves the activity was more during bearing period than pre bearing period. The variation in NR activity in leaves (both during pre-bearing and bearing period) and berries among the accessions was significant. Both during pre-bearing and bearing period, HP 1411 showed highest NR activity (940 and 1183 m moles of NO_2 g^{-1} fw^{-1} hr) while the lowest activity (440 and 593 m moles of NO_2 g^{-1} fw^{-1} hr) was recorded in Accn 840 during both the stages. Berry NR activity was highest in OPKM (264 m moles of NO_2 g^{-1} fw^{-1} hr) and lowest in Accn 4132 (38 m moles of NO_2 g^{-1} fw^{-1} hr). The mean NR activity in high yielders (768.0, 843.5 and 211.7 m moles of NO_2 g^{-1} fw^{-1} hr in leaves during pre-bearing period, bearing period and in berries respectively) was significantly higher (t test at 5 % level) than that of low yielders (595.8, 661.6 and 88.0 m moles of NO_2 g^{-1} fw^{-1} hr in leaves during pre-bearing period, bearing period and in berries respectively). There was a significant positive correlation between nitrate reductase activity and yield levels (r = 0.27, p = 0.03 and n = 66).

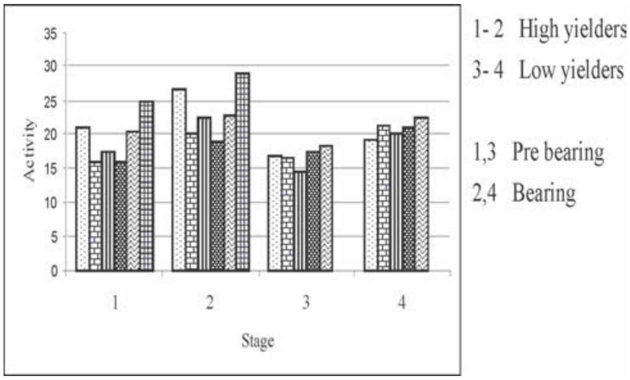


High yielders : Accn 1041, HP 780, OPKM, HP 813, Panniur 1 and HP 1411 in that order

Low yielders : Accn Nos 1157, 4132, 840, 1358 and 1535 in that order

Fig. 1. Nitrate reductase (NR) activity (m moles of NO_2 g^{-1} fw^{-1} hr) of high and low yielding black pepper accessions.

Sucrose phosphate synthase activity also differed significantly among the accessions both during pre bearing and bearing stages. But the mean activity of high (19.3 µ moles of fructose g^{-1} fw) and low (16.7 µ moles of fructose g^{-1} fw) yielders was on par during pre-bearing period (t test, non-significant). The activity was on par during bearing period also. The mean activity of high yielders was 23.3 µ moles of fructose g^{-1} fw and that of low yielders was 19.8µ moles of fructose g^{-1} fw during the bearing period. HP 1411 showed highest activity while accn 840 recorded lowest activity both during pre bearing and bearing periods. Figure 2 shows the SPS activity in high and low yielding black pepper accessions during prebearing and bearing periods.



High yielders : Accn 1041, HP 780, OPKM, HP 813, Panniur 1 and HP 1411 in that order

Low yielders : Accn Nos 1157, 4132, 840, 1358 and 1535 in that order

Fig. 2. Sucrose phosphate synthase activity (µ moles of fru ctose/g fw) as influenced by yield levels.

The productivity in black pepper is dependent on the formation of fruiting branches. The fruiting branch formed should accumulate adequate quantity of carbohydrates for spike production during the current season and also for the formation of laterals during the next season. The upper part of the canopy with a relatively higher leaf area during the spike development period and higher photosynthetic rate promoted the growth and development of productive laterals and sustained relatively large number of spikes (Mathai, 11). Based on his investigations, Mathai (11) opined that for higher productivity in black pepper, exposure of higher leaf area to light, accumulation of high dry matter in the fruiting branches before spike initiation in June, higher carbon fixation capacity and higher translocation of photosynthates from leaves to the developing berries are very important. In productive varieties like Panniyur-1 and Karimunda, more than 50 % of the radio-activity (uptake) was present in the adjacent spikes. Also, the berries of these varieties mobilized more photosynthates (Mathai, 11). Present study also supported the earlier findings that productivity is dependent on carbon fixation rates in black pepper. High yielders showed significantly higher photosynthetic rate than low yielders. Higher stomatal conductance must have helped the high yielders to fix more carbon resulting in higher photosynthetic rate than that of low yielders.

Positive correlation between photosynthesis and yield has been documented in many crop species. In rubber, clone PB 235 which showed higher yield than GT1 during both dry and wet seasons had slightly higher photosynthetic rate than the latter but there was very little difference in stomatal conductance and transpiration rate between the two (Conforto *et al*., 3). In cassava, average photosynthetic rate of upper canopy leaves was significantly correlated with root yield and harvestable biomass across environments, suggesting that selection for high photosynthesis might lead to high yields if combined with other yield determinants, such as leaf area duration, high harvest index and strong root sink (Tafur *et al*, 17; El Sharkawy *et al*. 6). In mung and urd beans, higher photosynthetic rate and higher Rubisco activity in the M2 plants and a lower glycolate oxidase and malate dehydrogenase activities are regarded as productivity promoting traits (Ignacimuthu and Babu, 9). Results of the present study are also in conformity with the above cited findings that selection for higher photosynthesis may lead to higher productivity in black pepper also if associated with higher stomatal conductance, lower leaf temperature and higher starch content during berry development period. But in Jarusalem artichoke, no positive correlation was found between leaf photosynthetic rate and tuber yield, although there were significant cultivar differences for both parameters. It is thus suggested that, rather than using photosynthetic parameters for screening, primarily assimilate translocation should be improved to increase tuber yield of new varieties (Soja *et al*., 16).

There was significant difference in NR activity among the black pepper accessions. The mean NR activity of high yielders was more than that of low yielders, some of the high yielders showed low activity while some of the low yielders showed high activity resulting in non-significant correlation between NR activity and productivity. Deckard *et al*., (4) reported that in six maize hybrids the nitrate reductase activity of the total leaf canopy showed a significant positive correlation with grain protein (kg N/ha) and grain yield. In wheat also such positive correlation between NR activity and grain yield is reported (Blackwood and Hallam, 1). In tea, there existed a significant positive correlation between green leaf yield and NR activity (Venkatesan and Ganapathy, 20; Wickremasinghe *et al*., 21). In okra, improved cultivars showed a higher rate of nitrogen translocation from leaves to fruits than local cultivars which had higher carbohydrate content during vegetative stage and higher NR activity in leaves (Singh, 15). But Traore and Maranville (18) reported that nitrate reductase activity did not correlate with grain yield or shoot biomass, but did correlate with grain N concentration in grain sorghum. In the present investigation also, total NR activity (leaves during pre-bearing/bearing stages + NR activity in berries) showed significant correlation with yield levels of the accessions studied.

In maize hybrids, SPS activity in the fourth leaf was significantly correlated with the forage dry matter yield measured in the field in 1991 suggesting that SPS activity could be a limiting factor of maize development; but no significant correlations were detected between SPS activities and forage yield measured the next year in two locations (Causse *et al*., 2). In tomato, one 35S line which maintained high leaf SPS activity throughout development yielded 70-80% more than controls at both normal and elevated CO_2 in open-top chambers in the field and 20-30% more than controls in two additional field trials. The other 35S line and the two SSU lines either yielded less or did not differ from controls under several growth conditions (Laporte *et al*., 10). In black pepper also though high yielders showed higher mean SPS activity it was not correlated with productivity. SPS may influence yield through carbon partitioning. Huber (8) is of the opinion that SPS may be involved in carbon partitioning between sucrose and starch. High yielders showed higher starch in berries compared to the low yielders during the initial berry development period. This may be due to higher berry carbon fixation or due to higher rate of partitioning of photosynthates during initial berry development period. Higher partitioning could be due to the higher sink drawing ability. Uma Shaanker *et al*. (19) reported that in Syzygium the dominant ovule will have higher sink drawing ability during the initial seed development period, thus accumulating more metabolites. Results of the present study also indicate that high yielders may have higher sink drawing ability than low yielders during the initial berry development period allowing them to accumulate more metabolites than low yielders. Increase in starch and amino acids content lead to an increase in tuber yield in potato (Regierer *et al*., 12).

To conclude, selection for high yielders at an early stage in black pepper may be achieved with seedlings having high photosynthetic rate, nitrate reductase activity and stomatal conductance and low leaf temperature besides other important features like high photosynthetic leaf area and less mutual shading *etc*.

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