SUCROSE AND NITROGEN EFFECTS ON GREENNESS, HEAD PARAMETERS AND FLOWERING OF FOUR LINES OF CABBAGE

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ABSTRACT

A study was carried out to determine whether increasing the sucrose concentration to seedlings in vitro and subsequently increasing N supply to plants in soil can affect head parameters or greenness which may lead to flower induction in cabbage. A further aim was to find out whether there is a relation between SPAD meter values for greenness and total chlorophyll of four cabbage varieties under study. Sterilized cabbage seeds were cultured individually in glass jars with heat sterilized agar medium containing 0 or 3 % sucrose. Plants generated were raised in 23 cm pots and a total of 0 or 7 g N per pot was applied as a top dressing to the plants. In a separate experiment, after greenness had been measured from leaves of each of the four varieties, 1 cm leaf discs were cut from the exact points where the measurements of greenness were taken and total chlorophyll determined for each disc. Sucrose and N increased the stem height at flowering independently and the combined effect of 3 % sucrose and 7 g N promoted early flowering of 'HRI 006556' as compared to when each treatment was applied alone. Supply of 7 g N caused head splitting of three varieties and reduced the solidity of 'KK Cross'. Variety, sucrose and N affected the SPAD readings. There were significant positive relationships between SPAD meter value for greenness and the total chlorophyll for data pooled from all the four cabbage varieties, however there were variety differences in the relationship that required the development of a separate regression equation for each variety of cabbage for effective prediction of total chlorophyll from SPAD meter readings.

INTRODUCTION

Cabbage (*Brassica oleraceae* var *Capitata* L.) is one of the most important and popular vegetable crops in the family, Brassicaceae. Cold treatment at temperatures in the range of 0^0-10^0C is required to induce cabbage to flower and subsequently produce seeds. The average minimum daily temperatures in most tropical countries are above the vernalization temperatures of cabbage and therefore cold is the major limitation to cabbage seed production in the tropics. Efforts are being made to find other ways of inducing flowering in cabbage without the normal green plant vernalization (Nyarko *et al.*, 2006, 2007) to facilitate cabbage seed production in the tropics.

Sucrose has been identified as a carbohydrate probably involved in vernalization (Friend *et al.*, 1984; Atherton *et al.*, 1987), while other authors found that nitrogen (N) influences flowering in brassicas (Gott *et al.*, 1955; Colder and Coope, 1961; Parkinson, 1952 cited by Hand, 1988). It was also observed in a previous experiment that two varieties of cabbage ('HRI 0011446' and 'HRI 006556'), which flowered without vernalization, showed a consistently high level of greenness as compared to the other varieties throughout the eight week study period (Nyarko *et al.*, 2006). Greenness (amount of chlorophyll in a plant) is closely related to the nitrogen content of the leaves (Anderson *et al.*, 1993). The aim of this experiment was to determine whether increasing the sucrose concentration to seedlings *in vitro* and subsequently increasing N supply to plants in soil can affect greenness or head parameters which may lead to flower induction in cabbage. A further aim is to find out whether there is a consistent relationship between SPAD meter values for greenness and total chlorophyll of the four cabbage varieties under study.

MATERIALS AND METHODS

Sucrose and nitrogen effect

Seeds of four cabbage varieties ('HRI 009617', 'HRI 006556', 'HRI 003202', and 'KK Cross') were separately dipped in 2 ml of 5 % Parozone bleach for 5 minutes. The seeds were then washed five times in 4 ml of purified water and cultured in 100-ml screw-capped glass jar (Beatson Clark and Co Ltd, Rotherham, UK) containing 15 ml of heat sterilized agar medium (Murashige and Skoog, 1962) with 0 or 3 % sucrose (weight / volume). The sucrose was added to the agar before autoclaving. Plants generated were transplanted into 4 cm-square plastic modular trays (filled with 46 g Levington F2S compost). When seedlings were 21 days old, they were transplanted into plastic pots of 23 cm diameter containing 3 kg Levington M2 soil mix. Then, 3.5 g N (ammonium nitrate) per pot was applied as a top dressing on two occasions, i.e. 30 days and 60 days after transfer of plants to the 23 cm pots (a total of 7 g N / pot). The control had no N top dressing. The experimental design was 4 x 2 x 2 factorial in a randomized complete block with four replications. In addition to the N treatment, all the plants had basal nutrients from Sangral compound fertilizer (1:1:3). Fifty (50) ml of a stock solution (100 g l^{-1}) were mixed with 5 l of water and applied to five plants. There were weekly applications for 12 weeks starting from 6 weeks after transplanting.

The following assessments were made:

Parameter	Explanation
Head length	Length of cut head longitudinal from base to tip
Head width	Width of cut head about mid portion
Head shape index	Head length/head width
Solidity	Head width / (0.523 x horizontal width x head length)
Days at flowering	
Stem height at flowering	

Greenness was also measured fortnightly from 14 d to 42 d after transplanting using a SPAD meter (Minolta Camera Company, Osaka, Japan) on two most recently fully expanded leaves of each of the plants. Five readings were taken from each leaf and the average SPAD reading of all leaves was computed for each plant.

Relationship between SPAD values and total chlorophyll

At 28 days after sowing, two leaves (of distinct different visual level of greenness) from the control and N treated plants respectively of each variety of cabbage were selected and three plants per variety were used. For each leaf, SPAD meter values for greenness were measured using a Minolta SPAD meter avoiding the mid rib. Leaf discs were cut (with 1 cm diameter cork borer) from the exact points where the measurement of greenness was taken. Each leaf disc was ground separately with a pestle and mortar in 1 ml of 80 % acetone (volume by volume) which was then made up to 4 ml of 80 % acetone in a Falcon tube. The tube and the contents were centrifuged at 3000g for 5 minutes. Absorbance was then measured at 645 and 663 nm using a Sp6-500 ultra-violet spectrometer (Pye Unicam, UK) with 80 % acetone as a blank. The total chlorophyll was determined using the following formula developed by Arnon (1946):

Total chlorophyll = $(8.02 \times A_{663}) + (20.21 \times A_{645})$

where, A_{663} and A_{645} are absorbance at 663 nm and 645 nm respectively.

Analysis of variance was used to determine the variation among cultivars for all parameters measured except proportions of head splitting where logistic analysis (Binomial) was adopted using Genstat-Release 8.1 statistical package. The data for the head parameters were square root transformed to normalize the error distribution before the analysis was performed.

RESULTS

Sucrose and nitrogen effect

Among the four lines, only one, 'HRI 006556', flowered. For this line, there was a significant interaction between the nitrogen and sucrose treatments ($P = 0.02$) on the days to flowering after transplanting (DFAT) (Table 1). Application of 7 g N reduced DFAT ($P = 0.02$) of the plants raised from medium with elevated (3 %) sucrose but not when no sucrose was added. None of the treatments or their interactions had effects ($P > 0.05$) on the girth and the number of leaves at flowering of the line 'HRI 006556', therefore that data is not presented. Sucrose and N increased the stem height at flowering ($P < 0.05$) of 'HRI 006556' independently; there was no interaction ($P > 0.05$) (Table 2).

The application of 7 g N as a top dressing significantly increased the proportion of splitting ($P = 0.004$) in the three lines of cabbage that did not flower (Figure 1). Line 'HRI 003202' appeared to be more resistant to head splitting than the other lines, with no splitting in the absence of N and the smallest proportion of splitting when N was applied.

Table 1: Effect of additional nitrogen and sucrose application on days to flowering after transplanting of line 'HRI 006556'.

Nitrogen	Days to flowering after transplanting	
	No sucrose	3 % sucrose
No additional N	12 (140)	13 (174)
7 g additional N	13 (157)	11 (118)
SED	0.735	
Sucrose x N interaction probability (P)	0.020	

Data were transformed (square root). Original scale data are in brackets.
SED = Standard error of the difference

Table 2: Main effects of additional nitrogen and sucrose on the average stem height at flowering for cabbage line 'HRI 006556'.

Treatment	Stem height (cm)	SED	Probability (P)
No additional N	5.55 (31)	0.418	0.032
7 g additional N	6.63 (44)		
No sucrose	5.59 (31)	0.418	0.043
3 % sucrose	6.59 (43)		

Data were transformed (square root). Original scale data are in brackets.
SED = Standard error of the difference.

Most of the head parameters measured were influenced by the line or addition of 7 g N independently (Table 3). Head width, length, shape and weight varied depending on the line, however, head length and weight also decreased ($P = 0.008$ and $P = 0.015$, respectively) with addition of 7 g N supply. 'KK Cross' seemed to be the best line in terms of head parameter; it had the highest head weight and width, and was more rounded.

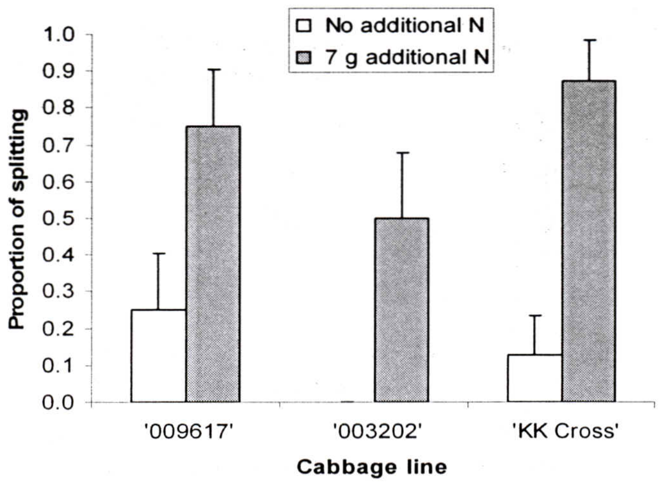

FIGURE 1: The proportion of splitting in three lines of cabbage in response to 7 g N supply. Bars show standard error of the mean proportion of splitting predicted from the regression model (n = 48, df = 36).

All the three and two way interactions (except that of the line x N) and the sucrose main effect were not significant ($P > 0.05$) as far as head solidity was concerned. The combined effect of line x N was therefore presented (Figure 2). There were no differences between the plants that were supplied with N compared with the control for all lines except KK Cross where additional supply of 7 g N reduced the solidity significantly.

Most of the combined effects of the three factors for SPAD meter value for greenness were not significant. The exceptions were line x date and nitrogen x date interactions (Figures 3a and 3b respectively). KK Cross consistently showed the lowest level of greenness from 21 d onwards while the rest

Table 3: Line and nitrogen main effects on head parameters

Line	Head (cm$^{1/2}$)	Head (cm$^{1/2}$)	Head shape	Head (g$^{1/2}$)
HRI 009617	3.409	3.966	1.168	24.33
HRI 003202	3.123	3.594	1.153	18.28
KK Cross	3.648	3.675	1.008	25.78
SED	0.0899	0.0754	0.0278	1.418
df	33	33	33	33
Probability (P)	<0.001	<0.001	<0.001	<0.001

Nitrogen	Head (cm$^{1/2}$)	Head (g$^{1/2}$)
7 g additional N	3.666	21.31
No additional N	3.824	24.28
SED	0.1066	1.582
df	33	33
Probability (P)	0.008	0.015

Data were transformed (square root) and transformed data presented.
SED = Standard error of the difference; df = Degrees of freedom

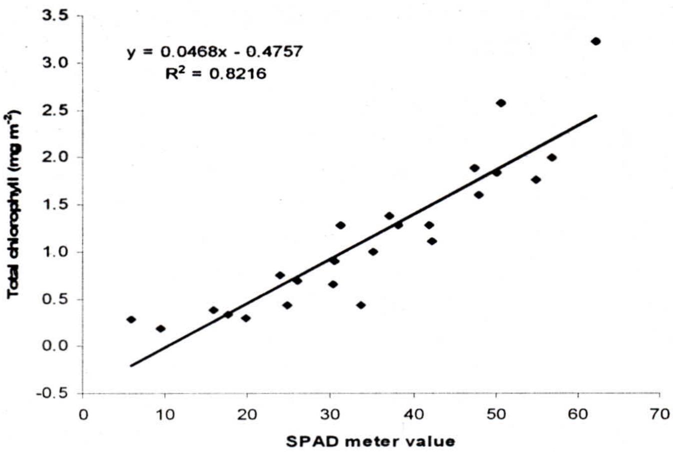

FIGURE 4: Linear regression for SPAD value for greenness and total leaf chlorophyll from four lines of cabbage.

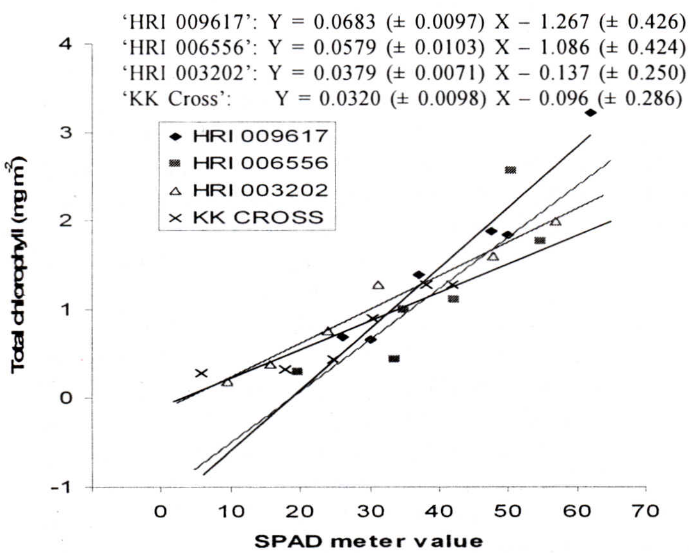

FIGURE 5: Linear regression for SPAD value for greenness and total leaf chlorophyll of four lines of cabbage.
Figures in brackets represent standard errors of the slope and intercept respectively.

vegetative growth before flower initiation and that may have accounted for the slight delay in flowering of either N or sucrose treated plants (Table 1). The reduction in number of days to flowering after transplanting (DFAT) of this line by the combined effect of sucrose and additional N as compared to when applied on their own is relevant. This line can flower without vernalization and is believed to undergo a vernalization-independent pathway which utilizes sucrose at the apical point to stimulate the plant to flower (Dennis *et al.,* 1996). N is also known to promote flowering in some plants (Parkinson, 1952 cited by Hand, 1988; Colder and Cooper, 1961). In winter rye, for example, Gott *et al.* (1955) showed that N has little or no effect at any stage of flowering in fully vernalized plants, but in unvernalized material a low N level may slightly retard progress to flowering. It was therefore not unexpected that the combined effect of the two factors promoted early flowering of the line as compared with when the treatments were applied independently. The results gave an indication that neither N nor sucrose alone was involved in the flowering of that line. It is probable that sucrose and other N related metabolites are transported to the stem apex to stimulate flowering. This assertion agrees with the recent findings of Bernier and Perilleux (2005), who identified sucrose and reduced N compounds as part of substances involved in the flowering of *Sinapis alba* (white mustard).

DISCUSSION

Sucrose and nitrogen effects

The plants supplied with either sucrose or N grew more vigorously and appeared greener than the control. Hence, it was not unexpected that treated plants of line 'HRI 006556' took more time for

Supply of 3 % sucrose and 7 g N increased stem height independently (Table 2) without affecting the number of leaves at flowering. Plants supplied with either additional N or sucrose were probably able to photosynthesize better than the control and accumulated more carbohydrate into the cells. This may have led to more rapid cell division and cell expansion resulting in increased stem height. Wien and Wurr (1997) indicated that under conditions marginal for induction of flowers in cabbage, stem elongation appears to aid flowering as well. Therefore, the increase in stem length due to the application of 3 % sucrose or 7 g N may have aided flowering in 'HRI 006556' to some extent.

Additional supply of 7 g N caused head splitting of all the three lines that did not flower (Figure 1). This implied that N led to rapid and continuous growth of the plants after heading. It is known that under favourable growing conditions, the inner leaves can exert sufficient pressure at maturity to cause head splitting (Dickson and Wallace, 1986). Although, head splitting is not a very good trait when a marketable head is required, it can be a good physiological phenomenon when it comes to seed production because it facilitates flower stalk emergence in vernalized plants, especially for lines that have a very dense head and require head splitting prior to flower emergence (Nyarko *et al.*, 2006). In addition, the N reduced the solidity of 'KK Cross' (Figure 2). Unlike the other two lines ('HRI 009617' and 'HRI 003202') which formed loose or small heads and unfolded later to allow flower stalks to elongate (Nyarko *et al.*, 2006), 'KK Cross' formed a very dense head and reduction in head solidity (head density) caused by additional N supply would be expected to facilitate easier flower stalk emergence. Supplying additional 7 g N may therefore be a good practice in seed production of 'KK Cross'.

It was observed that when the control plants were forming heads, the N treated plants were still forming wrapper leaves. Therefore, it is likely that photosynthate was diverted for wrapper leaf production instead of head formation for plants that were given additional 7 g N which resulted in the reduction of head length and weight. The complex structure of chlorophyll is made up of the four basic elements C, H, O and N while that of sucrose is made up C, H and O (Streitweiser and Heathcock, 1981). It is therefore likely that both the additional supply of 7 g N and 3 % sucrose helped in the formation of more chlorophyll that resulted in the increased greenness (Figures 3b and 3c).

Relationship between SPAD meter values and total chlorophyll

Comparison between the regressions of the four lines gave an indication that, in order to predict the amount of chlorophyll from SPAD readings, it is necessary to develop a regression equation for each line of cabbage. Other workers have earlier reported that SPAD meter value for greenness may depend on the cultivar (Westerveld *et al.*, 2004; Soval-Villa *et al.*, 2002; Abdelhamid *et al.*, 2003). SPAD meter readings are based on the ability of leaf chlorophyll to absorb light of specific spectral band, i.e. red band (around 650 nm) where absorption by chlorophyll is high and in the infra red (around 940 nm) where absorption is low (Minolta, 1989; Peng *et al.,* 1993). This has made other workers suspect that differences in leaf thickness may account for the variability of SPAD meter readings between genotypes (Campbell *et al.*, 1990; Peng *et al.,* 1993) and this assertion was confirmed when SPAD values adjusted for specific leaf weight (an index of leaf thickness) improved the prediction of rice leaf N from SPAD meter readings (Peng *et al.,* 1993). It is also known that SPAD meter readings vary greatly depending on growth stage (Campbell *et al.*, 1990; Peng *et al.,* 1993). Thus, the differences in growth stages of the four lines may also partly explain the different regression models for each line. 'HRI 003202' is early maturing, 'HRI 009617' and 'KK Cross' are late maturing and 'HRI 006556' is a non-heading line. This means that, at the time of measurements, the cabbage lines may have been at different growth stages which can lead to differences in SPAD readings and the corresponding leaf chlorophyll.

CONCLUSION

Sucrose and N increased the stem height at flowering ($P < 0.05$) of 'HRI 006556' independently without affecting the leaf number. The combined effect of 3 % sucrose and additional 7 g N promoted early flowering of 'HRI 006556' as compared to when sucrose was applied in isolation. Additional supply of 7 g N caused head splitting of three lines that did not flower and reduced the solidity of 'KK Cross'. SPAD meter value for greenness was affected by the combined effects of line x date and additional N x date with additional N supply increasing greenness consistently over almost all dates. *In vitro* sucrose (3 %) supplied to cabbage increased greenness independently. There were significant positive relationships between SPAD meter value for greenness and the total chlorophyll for data pooled from all the four cabbage lines, however, there were line differences in the relationship that demanded the development of a regression equation for each line of cabbage for effective prediction of total chlorophyll.

REFERENCES

Abdelhamid, M., Horiuchi, T. and Oba, S. (2003). Evaluation of the SPAD value in faba bean (*Vicia faba* L.) leaves in relation to different fertilizer applications. Plant Production Science, 6: 185-189.

Anderson, D., Bullock, D., Johnson, G. and Taets, C. (1993). Evaluation of the Minolta SPAD meter for on farm N management of corn in Illinois. *Fertilizer Conference Proceedings* (January 25-27), Illinois, 5pp.

Atherton, J. G., Hand, D. J. and Williams, C. A. (1987). Curd initiation in the cauliflower (*Brassica oleraceae* var. Botrytis L.). In: *Manipulation of Flowering* (Atherton, J. G., ed.) Butterworths, London, pp. 113-145.

Bernier, G. and Perilleux, C. (2005). A physiological overview of the genetics of flowering control. Plant Biotechnology Journal, 3: 3-16.

Campbell, R. J., Mobley, K. N., Marini, R. P. and Pfeiffer, D. G. (1990). Growing conditions alter the relationship between SPAD-501 values and apple leaf chlorophyll. HortScience, 25: 330-331.

Colder, D. M. and Cooper, J. P. (1961). Effect of spacing and nitrogen level on floral initiation in cocksfoot (*Dactylis glomerata L.*). Nature, 191: 195-196.

Dennis, E. S., Finnegen, E. J., Bilodeau, P., Chaudhury, A., Genger, R., Helliwell, A., Sheldon, C. C., Bagnail, D. J. and Peacock, W. J. (1996). Vernalization and the initiation of flowering. Cell and Developmental Biology, 7: 441-448.

Dickson, M. H. and Wallace, D. H. (1986). Cabbage breeding. In: *Breeding of Vegetable Crops* (Bassett, M. J., ed.). AVI Publishing, Westport. pp 395-437.

Friend, D. J. C., Badson, M. and Bernier, G. (1984). Sucrose promotion of flowering in *Brassica campestris* L. *cv* Ceres. Plant Physiology, 75: 1085-1089.

Gott, M. B., Gregory, F. G. and Purvis, O. N. (1955). Studies in vernalization of cereals. XIII. Photoperiod control stages in flowering between initiation and ear formation in vernalized and unvernalized pektus winter rye. Annals of Botany, 21: 87-126.

Hand, J. D. (1988). Regulation of Curd Initiation in the Summer Cauliflower. Ph.D. Thesis, University of Nottingham, UK.

Minolta Camera Company Limited (1989). Manual for chlorophyll meter SPAD 502. Minolta, Radiometric Instrument Division, Osaka, Japan. 22 pp.

Nyarko, G., Alderson, P. G. and Craigon, J. (2006). Towards cabbage seed production in the tropics. Ghana Journal of Horticulture, 5: 45-50.

Nyarko, G., Alderson, P. G. and Craigon, J. and Sparkes, D. L. (2007). Induction and generation of flowering in cabbage plants by seed vernalization, gibberellic acid treatment and ratooning. Journal of Horticultural Science and Biotechnology, 82, 346-350.

Parkinson, A. H. (1952). Experiments on vegetative and reproductive growth of cauliflower. *Annual Report, National Vegetable Research Station* (1951), Wellesbourne, Warwick, UK. pp. 38-51.

Peng, S., Garcia, F. V., Liza, R. C. and Cassman, K. G. (1993). Adjustment for specific leaf weight improves chlorophyll meter's estimate of rice leaf nitrogen concentration. Agronomy Journal, 85: 987-990.

Soval-Villa, M., Wood, C. W. and Guertal, E. A. (2002). Tomato leaf chlorophyll meter readings as affected by variety, nitrogen form and night time nutrient solution strength. Journal of Plant Nutrition, 25: 2129-2142.

Streitweiser, A. and Heathcock, C. H. (1981). *Introduction to Organic Chemistry.* 2nd edition, Macmillan, New York, 1258pp.

Westerveld, S. M., McKeown, A. W., Scott-Dupree, C. D. and McDonald, M. R. (2004). The assessment of chlorophyll and nitrate meters as field tissue nitrogen tests for cabbage, onions and carrots. HortTechnology, 14: 179-188.

Wien, H. C. and Wurr, D. C. E. (1997). Cauliflower, broccoli, cabbage and Brussels sprouts. In: *The Physiology of Vegetable Crops* (Wien, H. C., ed.). CAB International, Wallingford, Oxon., UK. pp. 511-55.