Vegetative and Reproductive Characteristics of Poinsettia Altered by a Graft-transmissible Agent

John M. Dole¹ and Harold F. Wilkins²

Department of Horticultural Science and Landscape Architecture, University of Minnesota, St. Paul, MN 55108

Additional index words. *Euphorbia pulcherrima*, graft transformation, apical dominance, branching, axillary branching, axillary inflorescence, free-branching, restricted-branching

Abstract. Poinsettia (*Euphorbia pulcherrima* Wind. ex. Klotzsch) cultivars were divided into free-branching and restricted-branching groups. Auto and reciprocal grafts were made among three free-branching cultivars, Annette Hegg Brilliant Diamond (BD), Annette Hegg Topwhite (TW), and Annette Hegg Hot Pink (HP), and two restricted-branching cultivars, Eckespoint C-1 Red (CR) and Eckespoint C-1 White (CW). when CR scions were grafted onto BD stocks, vegetative characteristics of branching pattern and leaf morphology of CR plants were altered when compared to the control graft combination CR/CR (scion/stock). Branching pattern was determined by pinching the scion above the 12th node and measuring axillary shoot length, diameter, and node number 30 days later. CR scions grafted onto BD stocks produced a plant very similar to BD plants when axillary shoot length and node number were compared. However, axillary shoot diameter and leaf morphology were intermediate between CR and BD plants. Changes were retained after two generations of serial vegetative propagation and are considered permanent. The reproductive characteristics of anthesis date, bract color, and cyathia cluster diameter were not influenced by the stock. CR/BD plants produced twice as many axillary inflorescences as BD/BD or BD/CR plants, while CR/CR plants did not produce any. All of the free-branching cultivars were able to alter the vegetative characteristics of all of the restricted-branching cultivars.

One of the most distinguishing characteristics of a poinsettia cultivar is branching pattern. Poinsettia cultivars are divided into two groups: free-branching, characterized by weak apical dominance and relatively small stem diameters; and restricted-branching, characterized by strong apical dominance and large stem diameters. Most of the cultivars grown today are free-branching and are from the Annette Hegg and Gutbier V-10 and V-14 cultivar series. The free-branching characteristic is important in production of vegetative cuttings and multiflowered plants. Free-branching cultivars produce the most axillary shoots and cuttings per plant when pinched.

While cultural factors such as light, nutrition, and temperature can temporarily alter branching capacity, other factors may permanently control branching. Preil and Engelhardt (1982) observed a decrease in branching in 80% of the plants regenerated from 'Annette Hegg Brilliant Diamond' suspension cultures. Plants with altered branching also had thicker stems, longer internodes, and more elongate and lobed leaves. Progeny of sexually propagated poinsettias exhibited reduced branching when compared to the parents (R. Stewart, personal communication). This phenomenon occurred even when both parents were free-branching cultivars. Researchers also noted that 5% to 10% of plants propagated from heat-treated free-branching poinsettias branched less than non-heat-treated free-branching poinsettias (R. Fulton, personal communication).

Stimart (1983) demonstrated that grafting promotes axillary shoot development when reciprocal grafts are made between

free-branching (self-branching) and restricted-branching (non-branching) poinsettias. Axillary bud growth was promoted on younger nodes of restricted-branching 'Eckespoint C-1 Red' when grafted onto free-branching 'Gutbier V-10 Amy', 'Gutbier V-14 Glory', or 'Super Rochford' Stocks. Branching capacity of restricted-branching cultivars was increased. Stimart also noted that the increase in branching was retained in cuttings propagated from 'Eckespoint C-1 Red' scions.

From the observations by Stimart (1983), Preil and Englehart (1982), and R. Stewart and R. Fulton (personal communications), the hypothesis was developed that branching capacity in the poinsettia is under two control systems: the genetic components that comprise a normal heritable system and a graft-transmissible free-branching agent that is heat and suspension-culture sensitive and is not transferred during sexual reproduction.

The objectives of this study are to verify the presence of a graft-transmissible, free-branching agent and to characterize its effects on vegetative and reproductive poinsettias.

Materials and Methods

The following materials and methods apply to all three experiments unless otherwise stated. Rooted cuttings were received from Paul Ecke Poinsettias (Encinitas, Calif.) and planted in 10-cm (450-ml) pots filled with a mixture of 3 sphagnum peat : 1 perlite : 1 mineral soil (by volume). Plants were grown in a glasshouse at a constant 24C. When needed, vegetative conditions were maintained by either natural long days (LD) or incandescent lamps as a 4-hr night interruption (2200–0200 HR). Short days (8 hr, SD) were provided by using black cloth (1600–0800 HR). Plants were placed in a completely randomized design.

Two grafting methods were used: 1) Splice graft (Hartmann and Kester, 1975); after grafting, plants were placed under in-

Received for publication 6 Aug. 1990. Minn. Agricultural Experiment Station, Scientific Journal Series, Paper no. 16,332. This research was supported in part by Paul Ecke Poinsettias and the Univ. of Minnesota Computer Center. The authors acknowledge Nancy Hinrichs' assistance with data collection. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

¹Assistant Professor, Dept. of Horticulture and Landscape Architecture, Oklahoma State Univ., Stillwater, OK 74078.

²Professor. Current address: Nurseryman's Exchange, 2651 Cabrillo Highway North, Half Moon Bay, CA 94019.

Abbreviations: BD, 'Annette Hegg Brilliant Diamond'; CR, 'Eckespoint C-1 Red'; CW, 'Eckespoint C-1 White'; HP, 'Annette Hegg Hot Pink'; TW, 'Annette Hegg Topwhite'.

intermittent mist and all axillary shoots were periodically removed from scion and stock. 2) Approach graft (Hartmann and Kester, 1975); two plants were placed in a single pot and approach-grafted when they reached sufficient size. Appropriate portions of scion and stock plants were removed 30 days after grafting. At that time, scions were pinched to three to four nodes above the graft, and grafted plants were placed under intermittent mist to reduce transpiration. After scion turgidity was reestablished, all axillary shoots were periodically removed from scion and stock except for the acropetal shoot on each.

A node was counted when the leaf had unfolded off the apical cone. A shoot was harvested as a cutting when at least two fully expanded and three to four expanding leaves were present. Cutting bases were treated with rooting powder containing 0.1% IBA (Hormex Rooting Powder No. 1, Brooker Chemical, N. Hollywood, Calif.) and rooted under intermittent mist in air at a constant 21C. The medium was at 24 to 27C, with supplemental bottom heat. Standard cultural practices were followed.

Annette Hegg and Eckespoint splice grafts (Expt. 1). The following scion/stock graft combinations were made on 23 and 24 Mar. 1985 using the splice graft method: BD/BD, CR/CR, BD/CR, and CR/BD. On 10 June 1985, 10 plants from each graft combination were randomly selected as cutting sources and pinched to nine nodes above the graft union. Vegetative cuttings, denoted as generation 1, were harvested from scions of the original grafted plants. Cuttings were rooted and randomly divided into two groups of 80 plants each (20 plants from each of the four graft combinations). One group was kept vegetative for the vegetative study, and the second group was placed under SD for the reproductive study. When the vegetative plants produced 16 nodes, they were pinched above the 12th node, and data were collected on axillary shoots 30 days later. Vegetative cuttings from these plants, denoted as generation 2, were harvested and treated similarly to generation 1 plants.

Data collected on vegetative plants were: axillary shoot length, diameter and node number per shoot, and leaf morphology observations. Data were averaged per node and analyzed by analysis of variance procedure for balanced data (SAS Institute, Cary, N.C.).

With the reproductive study, when generation 1 and 2 plants had produced 12 nodes, they were placed under SD. Data collected at time of anthesis were: cyathia cluster diameter, axillary inflorescence number, and days to anthesis. Data were not collected on plants that prematurely initiated flowers (split); thus, an uneven number of treatments resulted. Data were analyzed by the general linear model (GLM) procedure for unbalanced data (SAS Institute).

Annette Hegg combinations—approach grafts (Expt. 2). The following free-branching Annette Hegg cultivars were used: BD, HP, and TW. Fifteen grafts of each combination listed in Table 1 were made between 9 and 31 Oct. 1985 using the approach graft method.

Plants were placed under SD when the axillary shoot from the scion had produced 12 nodes. Bract colors of the scion and the stock were observed at scion anthesis.

Annette Hegg—Eckespoint combinations—approach grafts (Expt. 3). The following cultivars were used: BD, TW, CR, and CW. Ten grafts of each combination listed in Table 1 were made between 9 and 31 Oct. 1985 using the approach graft method. Not all of the grafted plants survived, resulting in an uneven number of treatments. Plants were placed under SD when the axillary shoot from the scion had produced 12 nodes.

Table 1. Graft combinations made in experiments 2 and 3 using free-branching BD, TW, HP and restricted-branching CR and CW poinsettia plants.

Experiment 2		Experiment 3	
Scion	Stock	Scion	Stock
BD	BD	BD	BD
BD	TW	BD	CR
BD	HP	BD	CW
TW	TW	TW	TW
TW	BD	TW	CR
TW	HP	TW	CW
HP	HP	CR	CR
HP	BD	CR	BD
HP	TW	CR	TW
		CW	CW
		CW	BD
		CW	TW

Bract colors of the scion and the stock were observed at scion anthesis.

Inflorescences and nodes with red- or white-pigmented leaves were removed after all plants reached anthesis on 28 May 1986. Axillary shoots were periodically removed, except for the acropetal shoot on the scion. When this shoot had produced 16 nodes, it was pinched above the 12th node and axillary shoots were allowed to grow.

Thirty days later, the number of nodes with axillary shoots >3 cm was recorded and leaf morphology was observed. Data were analyzed by the GLM procedure.

Results

Expt. 1. Data from generation 1 and from generations 1 and 2 combined were similar to those from generation 2 for the vegetative and reproductive studies. Thus, only data from generation 2 are presented.

Vegetative study. Numerous changes occurred in CR scions when grafted onto BD stocks as compared to the control graft combination CR/CR (Figs. 1, 2, and 3). CR/BD axillary shoots from the lowest eight to nine nodes elongated considerably, resulting in a significantly greater length, diameter, and node count than the CR/CR combination. Axillary shoots of the uppermost two to three nodes of the CR/BD plants were significantly shorter and thinner than shoots on CR/CR control plants, but node counts were similar. These changes were retained through two generations of serial vegetative propagation.

CR/BD axillary shoots had a significantly greater diameter at nodes 2, 8, 9 than the control BD/BD combination shoots (Fig. 2). The two combinations were not significantly different at any node for length and node count (Figs. 1 and 3).

BD/CR axillary shoots were significantly shorter at nodes 1 and 2, and node count at node 2 was lower than for control BD/BD shoots (Figs. 1 and 3). The two combinations were not significantly different at any node for diameter (Fig. 2).

CR/BD leaves were distinctly more rounded and less deeply lobed than CR/CR leaves, but less rounded and more deeply lobed than BD/BD or BD/CR leaves (Fig. 4).

Reproductive study. Cyathia cluster diameter and days to anthesis were not influenced by the stock (Table 2). The combination CR/BD produced twice as many axillary inflorescences as the BD/BD or BD/CR combinations, while the CR/CR combination did not produce any axillary inflorescences (Table 2).

Fig. 1. Axillary shoot length for generation 2 poinsettia plants originating from scions of auto and reciprocal grafts between free-branching BD and restricted-branching CR poinsettia plants. Data were collected 30 days after pinching plants above 12th node and means were an average of data from 20 plants. Vertical bars represent HSD ($P = 0.05$), Expt. 1. Original treatment scion/stock: ●—● BD/BD; ○—○ BD/CR; △—△ CR/BD; ▲—▲ CR/CR.

Fig. 2. Axillary shoot diameter from generation 2 poinsettia plants originating from scions of auto and reciprocal grafts between free-branching BD and restricted-branching CR poinsettia plants. Data were collected 30 days after pinching plants above 12th node and means were an average of data from 20 plants. Vertical bars represent HSD ($P = 0.05$), Expt. 1. Symbols as in Fig. 1.

Expt. 2. No visual changes were observed in bract color of shoots from either the stock or scion of any of the graft combinations listed.

Expt. 3. When CR or CW scions were grafted onto Annette Hegg stocks, at least 235% more axillary shoots were >3 cm than with Eckespoint/C-1 scions on Eckespoint C-1 stocks (Table 3). There was no difference between CR/CR and CW/CW combinations. There were also no differences among combinations that used Annette Hegg cultivars as a stock or scion. No visual changes were observed in bract color of shoots from either the stock or scion of any graft combination.

Discussion

Dramatic changes occurred to CR shoots after being grafted onto BD stocks (Figs. 1, 2, and 3). Apical dominance was

Fig. 3. Axillary shoot node number from generation 2 poinsettia plants originating from scions of auto and reciprocal grafts between free-branching BD and restricted-branching CR poinsettia plants. Data were collected 30 days after pinching plants above 12th node and means were an average of data from 20 plants. Vertical bars represent HSD ($P = 0.05$), Expt. 1. Symbols as in Fig. 1.

Fig. 4. Representative leaves of generation 2 poinsettia plants originating from scions of auto and reciprocal grafts between free-branching BD and restricted-branching CR poinsettia plants. Graft combinations noted as scion/stock. Expt. 1.

reduced, which resulted in increased axillary shoot elongation when the apex was removed. Acropetal shoots did not reestablish apical dominance over the basipetal nodes from which grew longer, thinner axillary shoots with more nodes. CR scions grafted onto BD stocks produced a plant very similar to BD when axillary shoot length and node count were compared (Figs. 1 and 3). However, axillary shoot diameter and leaf morphology were intermediate between CR and BD (Figs. 2 and 4).

While reproductive characteristics such as days to anthesis,

Table 2. Reproductive characteristics from generation 2 poinsettia plants originating from scions of auto and reciprocal grafts between free-branching BD and restricted-branching CR poinsettia plants. Data were collected 30 days after pinching plants above 12th node and means were an average of data from 13 to 18 plants, Expt. 1.

Original graft combinations		Cyathia cluster diam (cm)	Axillary inflorescences (no.)	Time to anthesis (days)
Scion	Stock			
BD	BD	3.4	1.3	53.3
BD	CR	3.8	1.0	52.8
CR	BD	3.0	2.6	63.2
CR	CR	2.9	0.0	61.6
Significance		***	***	***
HSD (0.05)		0.4	1.2	2.9

***Significant at $P = 0.001$.

Table 3. Number of vegetative axillary shoots longer than 3 cm on scions of auto and reciprocal grafts between free-branching BD and TW and restricted-branching CR and CW poinsettia plants. Data were collected 30 days after pinching plants above 12th node and means were an average of data from eight to 10 plants, Expt. 3.

Graft combinations		Axillary shoots > 3 cm. long (no.)
Scion	Stock	
BD	BD	11.3
BD	CR	11.6
BD	CW	11.4
TW	TW	11.8
TW	CR	11.8
TW	CW	11.5
CR	CR	4.8
CR	BD	11.5
CR	TW	11.7
CW	CW	4.3
CW	BD	11.4
CW	TW	11.7
Significance		***
HSD (0.05)		1.3

***Significant at $P = 0.001$.

cyathia cluster diameter, and bract color were not altered by grafting, the production of axillary inflorescences was increased in CR from 0 to 2.6 per plant when CR scions were grafted onto BD stocks (Table 3). CR/BD combination plants flowered ≈ 10 days later than BD/BD and BD/CR plants, which may have provided enough time for more axillary inflorescences to develop on CR/BD plants.

If axillary inflorescence development is considered vegetative branching under reproductive SD conditions, grafting influenced vegetative and not reproductive characteristics.

The changes cannot be related to the temporary influence of hormones or carbohydrates from the stock because vegetative characteristics remained altered for two generations of serial propagation. An agent apparently moved through the graft union and affected either the plant's genome or the expression of the genome.

Other researchers have reported genetic alteration through grafting. One of the earliest reports is from Glustschenko (1950), who described "vegetative hybridization" —the process of grafting two plants together to obtain a unique phenotype either from the grafted plant itself or from progeny of first or subsequent self-crossings of the scion. This process could alter many characteristics, such as fruit shape of tomatoes. Glustschenko (1950)

used the mentor method of grafting, whereby a scion was cleft-grafted to a stock and all but the uppermost leaves were removed to keep the scion dependent on photosynthates from the stock. The scion usually was a seedling much younger than the stock.

Similar confirmations of "vegetative hybridization" were made using solanaceae plants and the mentor method of grafting by Fajnbron (1953), Glavinic (1955), and Hirata (1980) using tomato; by Hirata et al. (1986), Kasahara et al. (1973), Ohta and Chuong (1975a, 1975 b), and Yagashita et al. (1961a, 1961b, 1964, 1985, 1986, 1987) using pepper; by Hirata (1979) and Hirata et al. (1986) using eggplant; and by Frankel (1956, 1962, 1970) using petunia. Rajki and Pal (1966) grafted immature fruit from one eggplant cultivar to another and found that $\approx 20\%$ of grafted fruits produced seeds that grew into plants with altered characteristics.

There has been a lack of agreement in the literature over proper terminology for the phenomenon described in the above reports. Glustschenko (1950) called it "vegetative hybridization"; Yagashita (1961a), "graft hybridisation"; Ohta (1977), "graftoduction"; and Pandey (1979), "graft transformation". We felt the term graft transformation is most accurate and least confusing.

Of special note is that Ohta and Chuong (1975a) carefully documented the traits transmitted during graft transformation in *Capsicum annum*. They reported an overall rate of variant occurrence of 0.84% in the first, second, and third generation self-crossed progeny of the scion. The genotype of each variant was determined by self-crossing and test crossing to both parents to check for the possibility of accidental contamination. Ohta and Chuong (1975a) concluded that contamination could not be responsible for the majority of the variants and that the rate of variant occurrence was too high for spontaneous mutation. In a companion study, Ohta and Chuong (1975b) grafted scions onto stocks that had been inoculated 1 month previously with broad-bean wilt virus. Progeny showed an overall rate of variant occurrence of 16.5%, compared to an overall rate of variant occurrence of 2.0% in the progeny from grafts between uninoculated scions and stocks.

There is also a reciprocal, but less obvious, effect of CR on BD (Figs. 1, 2, and 3). This effect is apparent only on the lower one to six axillary shoots. The BD/CR combination tended to have shorter axillary shoots with fewer nodes at the basipetal one to six nodes on the main stem than the control BD/BD combination (Figs. 1 and 3). Leaf morphology was not affected (Fig. 4). The cause of the reciprocal inhibition is unclear. Stimart (1983) noted a significant decrease in total length of axillary shoot growth on self-branching (free-branching) cultivars grafted onto nonbranching (restricted-branching) plants; inhibition may have been due to the graft union. In contrast, our data were collected from generation 1 and 2 plants vegetatively propagated from the scions of the original grafted plants.

Graft transformation has definitely occurred in the poinsettia. However, data differed from the previous reports of graft transformation in that a variant occurrence rate of 100% was obtained as compared to the highest reported rate of 16.5% (Ohta and Chuong, 1975b). Also, in our study, alterations were found on the original grafted plants, whereas in previous reports changes were most commonly found in the first, second, and third generations and only rarely on the grafted plants.

Branching in poinsettias appears to be under two systems of control, the normal heritable genotype and the graft-transmissible agent. This hypothesis is supported by the permanence of the changes induced by the agent, its sensitivity to heat treat-

ment and suspension culture, and the agent's inability to be seed transmitted (R. Fulton and R. Stewart, personal communications; Preil and Engelhart, 1982). The genotype that controls the base level of branching found in all poinsettia cultivars is not affected by heat treatment, suspension culture, or grafting, while a free-branching agent is responsible for the difference in branching between free- and restricted-branching poinsettia cultivars. The agent apparently does not follow traditional patterns of heredity and is affected by many of the same processes that affect pathogens, in particular, viruses. However, the nature of the agent that moved from BD to CR is unknown.

Literature Cited

- Fajnbron, B. 1953. Heritable changes in tomatoes under the influence of grafting (in Russian). Ak. Nauk. SSSR, Moscow, Inst. Genetiki. Trudy. 20:210-240.
- Frankel, R. 1956. Graft-induced transmission to progeny of cytoplasmic male sterility in petunia. Science 124:684-685.
- Frankel, R. 1962. Further evidence on graft-induced transmission to progeny of cytoplasmic male sterility in petunia. Genetics 47:641-646.
- Frankel, R. 1970. Genetical evidence on alternative maternal and mendelian hereditary elements in *Petunia hybrida*. Heredity 26:107-119.
- Glavinic, R. 1955. L'hybridation végétative comme méthode clans la selection des tomates. Proc. 14th Intl. Hort. Cong. 1:440-445.
- Glustchenko, I.E. 1950. Die vegetative Hybridisation von Pflanzen Verlagkultur und Fortschritt G.M.B.H., Berlin [Translated by W. Hoppner from Glustchenko, I.E. 1948. Vegetative hybridization in plants, Moscow (Russian)].
- Hartmann, H.T. and D.E. Kester. 1975. Plant propagation-principles and practices, 3rd ed. Prentice-Hall Englewood Cliffs, N.J.
- Hirata, Y. 1979. Graft-induced changes in eggplant (*Solanum melongena* L.) I. Changes of the hypocotyl color in the grafted scions and in the progenies from the grafted scions. Jpn. J. Breeding 29:318-323.
- Hirata, Y. 1980. Graft-induced changes in skin and flesh color in tomato (*Lycopersicon esculentum* Mill.). J. Jpn. Soc. Hort. Sci. 49(2):211-216.
- Hirata, Y., N. Yagishita, L. Ledoux, M. Thiry, and L. Piels. 1986. Graft-induced change in pepper and eggplant. Eucarpia (Zaragoza) p. 19-23.
- Kasahara, J., T. Nakamura, and Y. Yoneyama. 1973. Graft-induced variation in red peppers (in Japanese). Rec. Adv. Breeding 13:73-89.
- Ohta, Y. 1977. Hereditary changes induced by grafting and by DNA application and their implication in *Capsicum* breeding, p. 147-154. In: E. Pochard (ed.). *Capsicum 77*. Institut National de la Recherche Agronomique, Avignon-Montfavet, France.
- Ohta, Y. and R.V. Chuong. 1975a. Hereditary changes in *Capsicum annuum* L. I. Induced by ordinary grafting. Euphytica 24:355-368.
- Ohta, Y. and R.V. Chuong. 1975b. Hereditary changes in *Capsicum annuum* L. II. Induced by virus-inoculated grafting. Euphytica 24:605-611.
- Pandey, K.K. 1979. Overcoming incompatibility and promoting genetic recombination in flowering plants. N.Z. J. Bot. 17:645-663.
- Preil, W. and M. Engelhardt. 1982. *In vitro* separation of chimeras by suspension cultures of *Euphorbia pulcherrima* Willd. Gartenbauwissenschaft 47:241-244.
- Rajki, E. and G. Pal. 1966. Transplantation of a part of the berry fruit of *Solanum melongena* L. Acta. Agron. Acad. Sci. Hung. 15(3-4):301-309.
- Stimart, D.P. 1983. Promotion and inhibition of branching in poinsettia in grafts between self-branching and nonbranching cultivars. J. Amer. Soc. Hort. Sci. 108:419-422.
- Yagashita, N. 1961a. Studies on graft hybrids on *Capsicum annuum* L. I. Variation in fruit shape caused by grafting and the effects in the first and second seed generations. Bot. Mag. Tokyo 74:122-130.
- Yagashita, N. 1961b. Studies on graft hybrids of *Capsicum annuum* L. II. Variation in fruit shape caused by grafting for three successive generations and the effects in the progeny. Bot. Msg. Tokyo 74:480-489.
- Yagashita, N. 1964. On the induced hereditary variation in *Capsicum* by means of grafting. Bul. Fac. General Educ., Tokyo Univ. Agr. and Tech. 1:1-49.
- Yagishita, N., Y. Hirata, K. Okochi, K. Mimiura, H. Mizukami, and H. Ohashi. 1985. Characterization of graft-induced change in capsaicin content of *Capsicum annuum* L. Euphytica 34:297-301.
- Yagishita, N. and Y. Hirata. 1986. Genetic nature of bushy plant type in the variant strain-induced by grafting in *Capsicum annuum* L. Euphytica 35:17-23.
- Yagishita, N. and Y. Hirata. 1987. Graft-induced change in fruit shape in *Capsicum annuum* L. I. Genetic analysis by crossing. Euphytica 36:809-814.