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The Study of Stem Recutting and Aluminum-sulphate Application on the Some Morphophysiologic Traits in Rosa (*Rosa hybrid*a cv.illona)

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ABSTRACT

This reasear ch was conducted in agricultural f aculty of Tehran University in 2005 for evalu ation the effect of application aluminum-sulphate levels in preserva tion solution with stem recutting on longevity of *rosa hybrida* cv.Illona cut flow er.Cut stems of rosa th at produced in gr eenhouse fro m biennial mother stocks was placed under treatm ent of aluminum-sulphate solution with concentrations (100- 150- 200 ppm) and stem recutting in 2 levels (by cutting / without cutting).

Then in th is treated flowers, measured this tr ait include (longevity / a, b chlorophyll / water absorption content / relative wate r content / tot al protein / ac tivity of peroxidase and catalase enzymes in petals .The results showed that the different levels of aluminum-s ulphate had signific ant effect on senescen ce percent, chlorophyll conten t, relative water content and activity of an ti- oxidant en zymes.Also, stem recutting levels had si gnificant effect on s enescence p ercent, chlorophyll content, water absorption, relative water content, total protein, activity of anti- oxidant enzymes.

Obtained results from interaction effect of between application of aluminum-sulphate and stem recutting showed that application of alumi num-sulphate with 150 p pm together with stem recutting caused significan t incr easing in b chlorophyll, tot al chloroph yll, water re lative con tent and activity of peroxidase and cat alase en zymes, from this wa y caused decreasing in flower senescence percent.

Key Words: Rosa ,Illona ,aluminum sulphate ,stem recutting ,protein ,peroxidase ,katalase enzymes.

INTRODUCTION:

Rosa by scientific name *Rosa hybrida* L. is a p lant by a wide range from growth habitats in Asia, north – Africa, north – America, and Europ e.Roses Usuall y cu ltures for different utilization for ex ample pot plan t, ou tdoor plant and perspiring. But cut flow er production and its export had productive and economical value. Main areas of rose production in iran ar e Tehran, khoozestan, markazi, mazandaran an d esfahan that main aim of theirs cultivation is cut flower production.

Rose cultiv ars are ver y m uch and th ere are commercial cu ltivars include : sonia, illona, noblesse (white and yellow) and … in iran.Rose cut flower longevity is depend on pre and post harvest different factors such: environmental factors (light, temperature, humidity) and physiological (nutrition and h ormonal) factors. [1.2] .Senescence proc ess in cut flow er is an energetic pheno menon that includes activ ity of some prescripting gen es.This gen es, encodes involved proteins in stimulation of nutrient materials and also, synthesis and absorption ethylene and st ress reac tions. P roteolitic analyze had an essential role in development, ho meostatic and plant tissue s senescence [3].Water stress in rose with increasing of eth ylene bios ynthesis and decr easing of some enzy mes activity, causes that longev ity and relative water content decreases in leaves[4].

In evalu ation o f flower and s pathe color ch ange,water potential, tr anspiration and s olution solid material in Anthurium plant to improve the cut flower quality resulted that max effect is obtained b y preservation so lution on flower quality and longevity [5]. In study of effective factors in rose different cu ltivars longevit y, r esulted that longevity in most of rose cultivars increased with application of disinfected water and pot. Bu t if these cultivars are contaminated b y bacteria, flower s wilting occur earlier than assigned tim e [6] . With application of aluminum-sulphate (150 mg/l) in preservation solution in Eustoma grandiflorum cut flower by absorption increasing rather than contr ol plants could increase flower longevity and quality [7].By applica tion of ethionin (aluminum-sulphate) and su crose in preserv ation solution for rose cut flower cultivars, showed tha t longevity and without neck-bending had significant incr easing in first red, nobelesse and safir cu ltivars rather th an control[8]. Confir med that by app lication of 300 ppm al uminum- sulphate and 5% sucrose in preservation solutio n, longevity in rose cut flower cv.marin had significan t 2 day s increasing rather than contro l[9]. By ut ilization of aluminum-sulphate (200 to 400 ppm) together sucrose (1 to 2%) in preservation solution, cou ld increase po lianthes cut flower lo ngevity to 12 day s[10]. Prescence of bact eria in s tem cut ting plac e known as the reason of water absorption factor and longevity d ecreasing and with application of aluminum-sulphate in pr eservation solutio n, incr eased th e longevit y in rose cu t flower cv.sonia[11]. By the application of 1 to 4% glucose to gether 200 mg aluminum-su lphate in Preservation s olution, s howed that in ros e cu t flower cv.sonia, by means of the incre asing the trans piration and absorption con tent (op ening the stoma), longevit y increases and fl owers rem ain al ive[12]. B y th e treats the rose stems with aluminum-sulphate, showed that by means of incr easing the water tr ansmission and absorption, longevity increased more th an 7 day s r ather th an control[13]. In study about ch emical tr eatments on rose cut flower cv ,illona longev ity and quality r esulted th at aluminum-sulphate and thio-sulphateAg tr eatments, increases flower longevity and Co-chloride treatments and aluminum- sulphate in creases crop quality [14]. With application of alum inum-sulphate (1 to 300 mg/l) together calcium ch loride (1 to 300 mg /l) in pr eservation solution could promote cut flower l ongevity and color quality in gladiolus[15].

MATERIALS AND METHODS

This exper iment was conduc ted with goal of effect evaluation of aluminum-sulphate uti lization in differen t concentrations in preservation solutions together rose stem recutting on traits include: cut f lower longevity , chlorophyll con tent, wate r abs orbance, wat er rela tive content, total protein and anti-oxidant enzymes activity.

In this experiment, sam ples of rose cv .Illona that is provided in environmental s tandard and the same conditions, and was done b y application of aluminum-sulphate in 4 levels (0- 100- 150- 200 ppm) in preservation solution togeth er stem recutting factor in 2 levels (recutting and none-recutting) with 3 replicates in duration of 10 days.

Statistical d esign in this experiment was randomized complete design with 2 mentioned factors and finally data, Statistical anal yzed with M STAT-C s oft-ware and comparison of means was done by Dancan method.

1-measure method of Flower longevity:

Longevity trait was measur ed b y utilizatio n of submitted m ethod, and b y atten tion to trai ts such: flower wilting, f lower color change , petals num ber opening, bending of flower neck and flowers freshness that are du e to flowers with out senescen ce and measured o n base of percent (%)[16].

2-measure met hod of chlorophyll con tent (A, B , total):

For m easurement of chloroph yll cont ent us ed from Meidner method and with spectrophotometer set (model: shimadzu UV – 160A) in 662 and 644 nm wavelengths[17].

3-measure method of water relative content:

Water relative content on base of percent (%) and with submitted method by Lisi [18.19].

Water r elative c ontent perc ent = fresh weight – dr y weight / saturant weight / dry weight × 100

4-measure method of total protein content:

For this m easurement us ed from M ac Adam method[20] and with application of spectrophoto meter set in 595 wavelen gth and expr essed in base of m gr/g petals fresh weight.

5-measure method of peroxidase enzyme activity:

For measurement of this trai t, Mac Adam method[20] used and with application of spectrophotometer set in 475 wavelength in 60 second time. Also, was applied 20 mmol sodium phosphate buffer with PH=6 and 200 mmol Goicol as an electron giver and 30% H_2O_2 as an electron acceptor. Was expressed in base of unit/mg of protein.

6- Measure method of katalase enzyme activity:

Also, for measurement of kat alase en zyme a ctivity Chanes and Mahely metho d[21] used and b y spectrophotometer set in 340 wavelengths in 30 second time. Was applied 20 mmol s odium phosphate b uffer with PH=7 and 30% H_2O_2 as an e lectron ac ceptor. kata lase enzyme activ ity conten t expressed in b ase of u nit/mg of protein.

RESULTS

By attention to results th at ob tained from var iance analysis table (table 1) was showed that stem recutting had significant effe ct on all tra its exception al absorption content and total pro tein and causes significant difference.In ab out senescen ce trait, 200 ppm al uminum-sulphate solution showed significant differ ence with other treatments. Also, 150 ppm solu tion with con trol treatment showed significant d ifference. But between 100 ppm this solution and control doesn't show significant difference.In relation to A chlorophyll, 150 ppm solution had significant effect rather th an other treatm ents and increas ed this trait but rath er th an 200 ppm alu minum-sulphate solution doesn't show significant di fference.Application of 100 ppm aluminum-sulphate solution rather than control treatment had significant difference in about b chlorophyll. But b y increasing of utilizat ion of more than 100 ppm doesn't show significant diff erence.Also in about total chlorophyll, app lication of 150 ppm aluminu m-sulphate had significan t effect rather than other treatments but b y increasing the utili zed lev el to 200 pp m doesn't show significant diff erence.In relati ve water content trait, was applied 150 ppm aluminum-sul phate showed significant difference wi th other treatments but b y in crease th e utilized lev el m ore than 150 ppm didn't sho w significant difference with previous treatments whereas showed significant diff erence with control and 1 00 ppm treatments.In ab out solution ab sorption content, was used 150 ppm alumi num-sulphate ha d significant difference with contro l treatment but by increase to 200 ppm didn't show significant differen ce with other tre atments.Also in total protein content trait, was applied 150 ppm aluminum-sulphate h ad sig nificant d ifference wi th con trol treatment whereas didn 't show signifi cant diff erence with oth er treatments. By increase the application to 200 pp m, didn't show significant difference with other treatments.In about peroxidase enzyme activity in petals, 150 ppm aluminum-sulphate rath er than o ther treatments showed significant difference. Also, between control and 100 ppm aluminum-sulphate treatment th ere was signific ant difference.Catalaze en zyme a ctivity tra it in pe tals, 100 ppm aluminum-sulphate r ather than con trol treatment showed significant differ ence but between this trea tment and 150 ppm treatment th ere wasn't significant difference.In base of obtai ned results from varianc e analyze table (table2,3) was s hown that int eraction effe ct between 2 exp erimental fa ctors (s tem recutt ing and aluminum-sulphate in ab out relative water contents, b and total chlorophyll, total protein, catalaze and p eroxidase enzymes act ivity tr aits was signific ant.In about rela tive water content trait, max of si gnificant differ ence was obtained from a $_1b_4$ and a $_2b_1$ treatments and m in of that related to contro l and a $_1b_2$ treatment. In otherwise, in 100 ppm aluminum-sulphate treatm ent the re was s ame effe ct to stem recutting and in a_1b_2 and a_1b_3 treatments, there was same effect to control in ab out this trait.In about B chlorophyll tr ait was obtained the max of significant difference from a $_2b_2$ treatment and min of t hat was obtained from control and a_1b_4 treatments.

Also in total chl orophyll, significant difference relates to a_2b_2 and a $_2b_1$ treatments and min significant difference was obtain ed fr om control and a $_1b_3$ tr eatments.In tot al protein trait max of signifi cant differen ce was obtain ed from a $_1b_3$ treatment and a $_2b_2$ and a $_2b_3$ treatm ents there is after its. Also min of that was obtained f rom a2b4 treatment and so, control tr eatment was placed after its.Max and min of significan t difference in peroxidase enzyme a ctivity rela tes to a $_2b_3$ and contro l tr eatments, respectively.Max and min of significant dif ference in catalaze en zyme activ ity tra it, re lates to a $_2b_3$ an d control treatments. Alth ough ther e wa sn't significant difference between a $_2b_3$ tr eatment with a $_2b_4$ and a_1b_4 treatments.In about traits such: longevity and a chlorophyll, there wasn't significant interaction effect between various treatments of aluminum-sulphate and stem recutting.

Table 1. was ev aluated traits variance analysis results in petals

S.O.V	df	Senescence	Achlorophyll	Bchlorophyll	Total chlorophyb	Absorption	Rwc	Total protein	Peroxidase	Catalase
		M.S.	M.S.	M.S.	M.S.	M.S.	M.S.	M.S.	M.S.	M.S.
Cutting(A)	1	158 *	11/7**	0/78*	10/7*	9/4 ns	60/2*	0/17 ns	0/001**	0/001**
Sulphate(B)	3	264**	3/6*	3/6**	2/2**	248 *	184**	2/5**	0/003**	0/001**
levelAB	3	733*	0/22 ns	0/94**	0/20*	587 ns	32/1*	0/33 ns	0/001**	0/001**
Error	16	294	0/34	0/15	0/07	660	9/8	0/15	0/001	0/001
Cv(%)	-	33/8	2/77	6/75	0/85	9/89	4/8	9/0	4/8	17/4

**:significant difference in 1% level *: significant difference in 5% level ns:insignificant difference

Table 2. w as s tudied tr aits means com parison b y dancan test in aluminum-sulphate different levels

Sulphate (μ ml^{-1})	Senescence (%)	Achlorophyll (mg/gFw)	Bchloro (mg/gFw)	Total chloro (mg/gFw)	RWC (%)	Absorption (cc)	Total protein (mg/gFw)	Peroxidase (U mg/protein)	Catalase (U mg/pro)
0 (b1)	78a	20/2b	5/2b	30/2b	9/2b	236b	4/1b	0/031d	0/024b
100 (b2)	54ab	20/4b	6/3a	29/7	62/0b	255b	4/6b	0/067b	0/035a
150 (b3)	40b	21/9a	6/6a	31/2	68/3a	286a	5/1a	0/081a	0/037a
200 (b4)	30c	20/9a	5/6ab	30/7	71/2ab	262ab	4/4ab	0/068b	0/031ab

Means with the same letter in each letter are not significantly different.

Table 3 was studied tr aits m eans com parison b y dancan test in cutting diff erent leve ls with aluminum-sulphate

Cutting (A) num-sulphate (B	tive content (%)	hlorophyll ng/gFw)	Total lorophyll ng/gFw)	al protein ng/gFw)	idase enzym it/mg pro)	ase enzyme it/mg pro)
b_1b_1	56d	4/9c	29/6de	3/9cd	0/026e	0/014d
A_1b_2	59d	5/7bc	29/8cd	3/8cd	0/055c	0/032b
A_1b_3	57d	6/5ab	29/2e	5/2a	0/073b	)/038ab
A_1b_4	71a	5/5c	30/7b	4/8ab	0/062c	)/036ab
A_2b_1	73a	5/4c	31/7a	4/4bc	0/036d	0/025c
A_2b_2	65bc	6/8a	31/6a	4/5ab	0/078b	)/034ab
A_2b_3	61c	6/9a	30/5bc	4/8ab	0/089a	0/039a
A_2b_4	69ab	4/8c	30/8b	3/3d	0/043d	)/035ab

Means with the sa me lette r in eac h letter are not significantly different.

DISCUSSION

By attention to obtained res ults from aluminum-sulphate solution treatments can results that b y increasing of utiliz ation o f alum inum-sulphate, flower senescen ce percent d ecreased and theirs lon gevity increased. At any rate whatever us age con tents in creases (to 200 p pm), the longevity tr aits increases,also.Reason of this phe nomenon is m ore water absorbance that resultin f of two characteristicsof anti-ba cterial and to settl e o f solution colloids aluminum-sulphate[22].

Also, Van door n [11] and Edrisi [14] were reached in their research to similar results. In other side, its reason is the effect on rel ative water cont ent that i t is due to flower turgor and viability . So, uti lization of alum inum-sulphate to 150 ppm fro m way of x ylems disinfection an d to settle of solution kollo ids causes to wa ter absorption in creasing and so cellul ar activ ation an d eth ylene bio synthesis decreasing and viabi lity increasing. s uch res ults were obtained by An derson [4] and St ater [12] and He.shenggen [23]) in thei r r esearches.Also, agree to Van d oorn[11] opinion, turgor includes cells viability and ac tivity that due to absorption and repelling and activity of one organism or cell. So, alumin um-sulphate application to 200 ppm causes chloroph yll co ntent in creasing from the way of ce ll a ctivation.Chlorophyll incr easing i s due to cells activity and to incr ease th e carboh ydrate production that carbohydrate in creasing causes flower s enescence decreases. Sim ilar result was o btained b y Gowda[10] ; Terril A [24] ; Ahn-ky[9] in their experiments.Aluminum-sulphate utilization to 150 ppm causes to incr ease katalase and perox idase enzy mes activity conten (anti-oxidant enzymes).This i ncreasing is due to cells a ctivation from way of suitable nutrient solution absorption and cell turgor. Cellular activation is one reason for activation of anti-oxidant enzymes and so c ells membrane hardiness so, inhibits from eth ylene bios ynthesis and outer factors damages and causes decreasing in H_2O_2 active species that was obtained f rom H_2O_2 analysis, from wa y of anti-oxidant enzymes activation. Such results were obtained by Williamson [25]; respec tively in bean and m aize, which can result in creasing of anti-o xidant en zymes activ ity, causes flower senescence de creases. Appli cation of aluminum-sulphate is effe ctive in ant i-oxidant enz ymes activity and th is topic is relat ed to flowers lasting and freshness in contrast H_2O_2 des truction. Beca use when flower shoots was separated fro m plants and preserved in solution, to be involved in stress specially water stress and in such conditions to establish anti-oxid ant enzy me activity.This su bject was ex perienced b y Xiao zhong and Huang [26] in grass plants from way water stress. Whereas free ox ygens that obtained from H_2O_2 anal ysis are one o f the im portant f actor in pe tals earl ier s enescence and in other side perox idase and ka talase enzymes are from anti-oxidants that causes poisonous effect natur alization of H_2O_2 free ox ygens. So, activity of these enzymes inhibits from petals s enescence[26.15].Aluminum-sulphate utilization tog ether stem recu tting in m ore contents showed significant differen ce on relative water content, B chlorophyll, to tal ch lorophyll, total pro tein, p eroxidase and kata lase e nzymes ac tivity rather than n on- stem cutting case th at this inter action effect can be effect ive indirectly on flowers freshne ss in c ontrast stre sses a nd other s enescence aggr avator factors .Stem recutt ing approximately is sim ilar to aluminum -sulphate utili zation without cutting and has same effect frow way of absorption in creasing in r elativw water con tent.Stem recutting is eff ective on m ost traits d irectly and has in some of them indirect effect. Such result was obtained by in Alstroem eria.What is im portant is tha t showed significant diff erence in most traits in cludes 150 ppm aluminum-sulphate together stem recutting.

In about relativ e water c ontent, 200 ppm aluminum-sulphate content is similar to stem recutting which its main reason is re lated to absorption conten t .Also, stem recutting togeth er 200 ppm aluminum-sulphate is placed in furth er grad e. Such result was obtain ed in ro se,and in Alstroemeria which m ay be i ts reason is re lated to evaporation and trans piration decreasing or due to water absorption ex cess b y ros e cut fl ower. In firs t c ase, ca b y the effect on water absorption with vasculars from the way of inhib iting fr om m icrobes growth and st em rotting causes to decre ase pet als s enescence.Agree to vandorm[11] b y decreasing the vasculars pollution content, wil l inc rease wat er abs orption and will decre ase senescence.In about B chlorophyll, stem recutting together 100 ppm, 150 ppm aluminum-sul phate solution s has max effect and shows signific ant difference. To increase in aluminum-sulphate utilization content hasn't excess effect in this trait whi ch sam e results was obtained b y Ter ril [24]. Also, in about total chlorop hyll, aluminum- incr ease of sulphate application cont ent has significant effect but when thes e two factors caus e t o act togeth er, especially stem recutting together 100pp m, 150 ppm aluminum-sulphate has max effect and shows significant difference. In total pro tein trait aluminum-sulphate ap plication without stem recutting is eff ective and shows significant difference altho ugh when stem recutting caus ed to act specially in 100 ppm; 150 pp m contents has max effect and shows signific ant diff erence.This result is agree to Gaspar [15] an d Terril [24] results.In about p eroxidase enzyme act ivity, increasing of aluminum-sulphate application content, h as signifi cant eff ect but w hen thes e two factors c aused to act together, es pecially s tem recutting with 1 00 ppm, 150 ppm aluminum-sul phate h as max effect and shows significant diff erence. This result agrees to Gas par [15] idea.1 and 10 µm c ytokinin utilization causes signific antly to in crease pr otein and peroxidase enzyme activ ity r ather than contro l and less application con tent (0/1 µm). Whereas was ob tained fr ee oxygen from H_2O_2 is one of i mportant factors in earlier petals senescence and peroxi dase enzy me is one of th e anti-oxidants w hich neu tralizes the poisonous effect of was obtained fr ee ox ygen from H_2O_2 and so inhibits from petal s enescence.Max of c atalaze activity content was obtained when 10 µm ol cytokinin utilized, by attention to same rol e of this e nzyme with peroxidas e enzy me in neutralization of free ox ygen t hat obt ained fro m H_2O_2, appears tha t increase th e c ytokinin utili zation level is related to c atalaze ac tivity in creasing and rol e of th is enzyme has an effect on p eroxidase en zyme activity and protection from cells against poisonous effect of H_2O_2 that at le ast is effe ctive signific antly in p etals senescenc e percent decreasing.

The results of Luhava et al 2003 experiment, is resemble to our experiment. So, the reas on of s enescence delay or longevity incr eases because utilizat ion of ca is related to increase the relative water content percent that is a reason for cells turgor incr easing and petals longevit y increasing or application of ca and decr easing of electrolyte s eeping bec ause of cel ls wall d estruction decreasing in petals that causes longevity to increase.

Also increas e the ca ut ilization causes to inc rease the anti-oxidants en zymes ac tivity (catalaze and pe roxidase) that causes fr ee ox ygen that obtain ed fro m H $_2O_2$ neutralized and finally senescence is decreased.

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