J. Agric. Sci. Mansoura Univ., 29 (12): 7229 - 7240, 2004

STUDIES ON JOJOBA [*Simmondsia chinensis* (LINK) SCHNEIDER]:

IV. PREFLOWERING SEX IDENTIFICATION

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ABSTRACT

For the prediction of jojoba seedling sex before flowering, some leaf shape parameters and indices were measured and calculated from known sex population of jojoba. Leaf shape indices included the basic parameters of leaf blade length (L), blade width at its one-fourth point from the base (W1), blade width at its widest region (W0) and the blade width at its three-fourth point from the base (W2) in addition to six suggested derived ratios which included: L/W0, W1/L, W0/L, W2/L, 2W1/L and 2W2/3L. It was statistically found that only six out of the 10 selected leaf parameters could be used for the identification of jojoba seedling sex (L, W2, W0, W1, W1/L and 2W1/L). The standard values of leaf parameters of jojoba shrubs indicated the possibility of using leaf morphological parameters to identify male and female jojoba plants.

Preflowering sex identification was carried out using three methods:

1- Preflowering sex identification by the confidence limits comparisons; the confidence limits of the different leaf parameters of the standard known sex jojoba shrubs were first calculated, data of the unknown seedlings were compared with the confidence limits of the standard known sex population. Generally, it was found that the different leaf parameters were able to identify the seedling sex of a limited portion of the population size, not more than 21.52% in the case of 2W1/L.
2- Preflowering sex identification by the overall mean comparisons; Since the means of males were always smaller than females, the seedling mean of any parameter was compared to the corresponding standard overall mean of that parameter. It was generally found that the different leaf parameters were able to identify successfully the sex of about 53% (W1/L) to almost 66% (W1) of the unknown population of jojoba seedlings.
3- Preflowering sex identification by the multiple separation method; it was possible by using the multiple separation method to identify accurately and high reliably the sex of 70 unknown jojoba seedlings (44.30% of the tested population), 22 males and 48 females.

Finally It can be strongly recommended to use the overall mean comparisons not only due to its extremely easy way of calculations but also to the highly accurate results of sex identification as well as the high percentage of identified seedlings obtained.

INTRODUCTION

Jojoba [*Simmondsia chinensis* (Link) Schneider, family Simmondsiaceae] is an evergreen oil producing shrub which gained prominence during the late 1970s when it was promoted as a substitute for sperm whale oil (Milthorpe and Dunstone, 1996). The sex of young jojoba plants can not be judged until the first flower buds appear which may happen after many years (Gentry, 1958 and Hogan, 1979). Lloyd and Webb (1977), have well reviewed the relationship between sex and the plant morphological characters. Sex differences in habit or plant shape have been reported in several dioecious trees. Female trees have

broader crowns in Gingko biloba, Juniperus communis and the Lombardy poplar. In *Taxus baccata*, male trees have taller growth and longer internodes. The leaves of male plants of *Mercurialis perennis* are narrower and smaller than those of females and in early spring are directed upwards and crowded near the top of the shoot, while those of females are fully expanded and spreading outwards. In *Silene alba*, the leaves (and the petals) of females are larger and have a higher length : width ratio than those of male plants. Wallace and Rundel (1979) reported that there is a difference in leaf size between male and female jojoba plants collected in Southern California and Mexico.

Kitat et al., (1980) successfully identified a considerable percentage of gametic citrus seedlings using different leaf shape indices. Chen *et al.*, (1985) tested three different methods to determine the sex of preflowering–stage young jojoba seedlings. These three methods were first developed with known sex populations as models. They are (1) the leaf length/ width (L / W) ratio, (2) Popov's colour test and (3) immunological assays. It was observed that the L / W ratio of all females is greater than the average L / W ratio of males. Lovenstein (1985) reported that preflowering sex determination of jojoba seedlings could possibly be done by leaf morphology since leaf measurements of male and female shrubs differ.

Jojoba (*Simmondsia chinensis*), as a dioecious desert shrub, is dimorphic in secondary sexual characters in some desert populations, where females on average have larger leaves and more open canopies than males (Kohorn, 1994). The reproductive behaviour of individuals tended to be associated with different morphological attributes for both males and females.

Helally (1999) has also successfully used the multiple separation method to identify zygotic and nucellar seedlings resulted from the seeds of the tested *Citrus* cvs. and hybrids by using different leaf morphological indices. Suganuma (1999) used starch gel electrophoresis to study peroxidase and esterase isozymes in leaf extracts from adult male and female shrubs and young seedlings of *Simmondsia chinensis*. The results showed that peroxidase and esterase zymograms could be used to identify the sexes of young seedlings.

Female plants of several dioecious angiosperms are commercially valued for production of fruits or seeds, such as papaya, pistachio, kiwi and jojoba. To make the cultivation profitable, it is necessary to grow more female than male plants. (Parasnis *et al.*, 2000). To discriminate between male and female plants, sex-specific molecular markers have been identified in a few dioecious species such as Silene and pistachio (Kafkas *et al.*, 2001). However, accurate and convenient sex diagnostic methods for early sexing of seedlings are not available to date. Due to the difficulties confronting jojoba growers with regard to identifying male and female young plants before plantation establishment, the main aim of this study was to find an easy and reliable method for the preflowering identification of seedling sex in order to obtain a good distribution of male and female shrubs, with proper ratio, in the field to get the highest possible yield per unit area.

MATERIALS AND METHODS

This experiment was carried out throughout the period of 1998 -2002 at the Research Stations of the Desert Development Center, The American University in Cairo. For the identification of jojoba seedlings sex, some leaf-shape parameters and indices were measured and calculated from known sex

populations of jojoba shrubs of about 20 years old. The male standard parameters were taken from three clones grown at Sadat station (SM1, SM3 and SM4) whereas the female standard parameters were taken from three clones grown at Tahrir station (SF1, SF2 and SF3). Leaf shape indices (modified after Kitat *et al.*, 1980 and Helally, 1999) included the basic parameters (in cms.) of leaf blade length (L), blade width at its one-fourth point from the base (W1), blade width at its widest region (W0) and the blade width at its three-fourth point from the base (W2) in addition to six suggested ratios which included : L/W0, W1/L, W0/L, W2/L, 2W1/L (tan angle1) and 2W2/3L (tan angle 2) as shown in Figure 1.

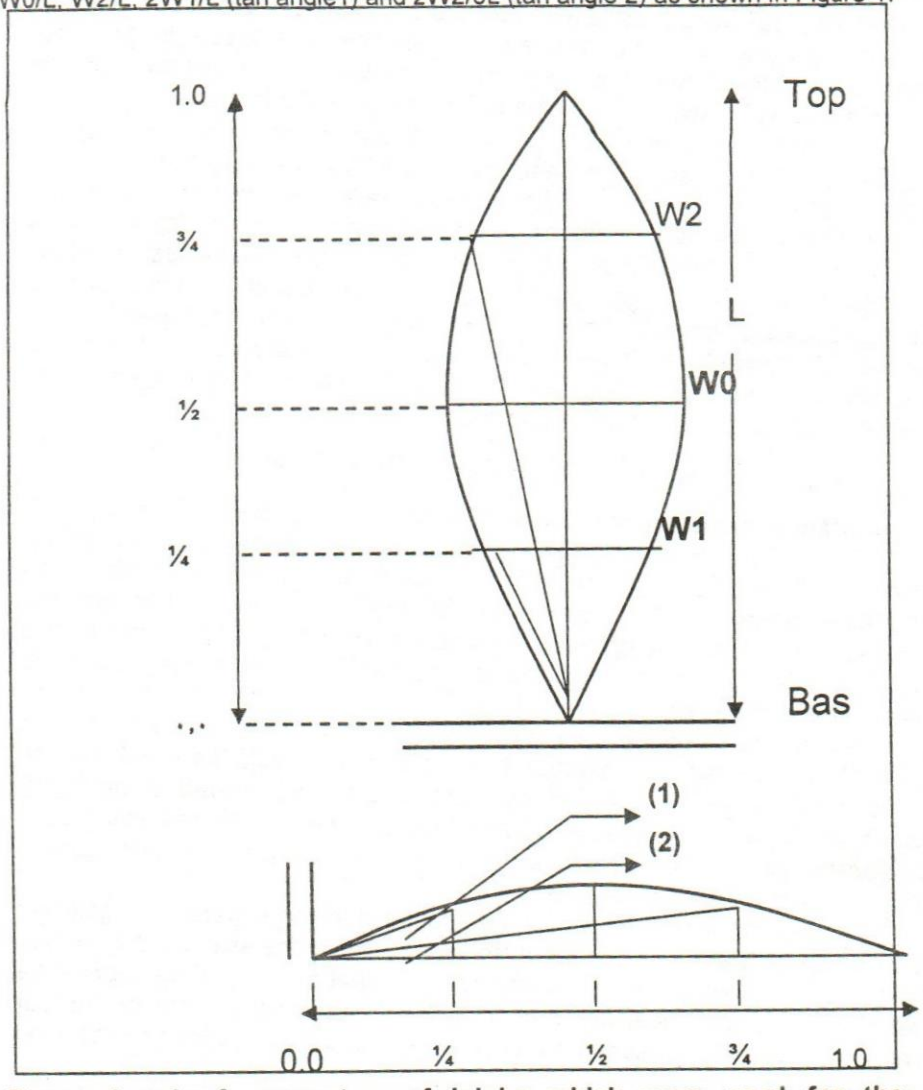

Figure 1 : Leaf parameters of jojoba which were used for the preflowering sex identification.

(L) = The leaf blade length. (W1)=Blade width at its one-fourth point from the base.
(W0) = Blade width at its widest region.
(W2) = Blade width at its three-fourth point from the base.
(1) = Angle (1) [Tan angle (1) = (2W1 / L)]. (2)= Angle (2) [Tan angle (2) = (2W2 / 3L)].

In order to calculate the standard leaf shape parameters, four pairs of leaves were measured (from the third to the sixth pairs of leaves from the branch top), the first and second leaf pairs were eleminated. A total of 120 branches were used to measure the different leaf shape parameters, 20 branches were taken from each parental shrub. The obtained data were then statistically analysed as a Factorial in Completely Randomized Design (Snedecor and Cochran, 1990). Factors used were : sex (two levels; male and female), leaf pairs (four levels; four pairs of leaves, the average of each pair was considered). The analysis of variance was made to test the significance of the different leaf parameters as well as to select the suitable pair of leaves which will be used as a source of these parameters.

In order to identify the sex of unknown eight months old jojoba seedlings, the leaf parameters and indices of 250 unknown sex seedlings collected at random (sown on October 1998) were measured and scored as an average of four pairs of leaves (from the third to the sixth pair of leaves from branch top). The 250 seedlings were kept in the greenhouse for about four years. All the seedlings were kept under regular observation, as soon as the plant flowers, the sex was recorded. After four years, 158 plants have successfully flowered, their sex became known. The leaf parameters of these 158 plants (taken since they were eight months old) were compared to the real sex regarding the type of flowers they have.

RESULTS AND DISCUSSION

1. Standard parameters of male and female jojoba shrubs :

A group of six jojoba shrubs of about 20 years old were used for measuring and calculating the standard parameters of leaves (three males and three females). The analysis of variance was made first to test the significance of the different leaf parameters, i.e. the practical comparative importance of each of them to identify the sex of jojoba plants, second to select the suitable pair of leaves which will be used as a source of leaf parameters. The obtained data are presented in Table 1. It was found that only six out of the 10 selected leaf parameters could be used for the identification of jojoba seedling sex. Statistically, they proved to be highly significant (L, W2, W0, W1, W1/L and 2W1/L), while the ratios L/W0, W0/L, W2/L and 2W2/3L were excluded since they were found not significant in distinguishing males from females.

As for the leaf pairs, it was found that there were no significant differences between the leaf pairs (from the third to the sixth pairs of leaves from the branch top). This gave an indication that any pair of them could be used as a source of leaf parameters. Nevertheless, the average of the four pairs was used for subsequent identification steps to get more accurate and reliable parameters.

The standard mean values of the different leaf parameters of male and female jojoba shrubs were calculated and presented in Table 2, which obviously indicated the possibility of using leaf morphological parameters to identify male and female jojoba plants.

Table 1: Analysis of variance of the different standard leaf parameters of male and female jojoba shrubs.

S.O.V.	d.f.	M.S.									
		L	W2	W0	W1	L/W0	W1/L	W0/L	W2/L	2W1/L	2W2/3L
Sex	1	1.835**	0.428**	0.547**	0.794**	$0.042^{N.S.}$	0.018**	$0.004^{N.S.}$	$0.004^{N.S.}$	0.074**	$0.000^{N.S.}$
Leaf pairs	3	$0.040^{N.S.}$	$0.015^{N.S.}$	$0.013^{N.S.}$	$0.005^{N.S.}$	$0.009^{N.S.}$	$0.000^{N.S.}$	$0.000^{N.S.}$	$0.000^{N.S.}$	$0.000^{N.S.}$	$0.001^{N.S.}$
Sex × l. pairs	3	$0.049^{N.S.}$	$0.007^{N.S.}$	$0.014^{N.S.}$	$0.014^{N.S.}$	$0.007^{N.S.}$	$0.000^{N.S.}$	$0.000^{N.S.}$	$0.000^{N.S.}$	$0.000^{N.S.}$	$0.001^{N.S.}$
Error	112	0.136	0.027	0.039	0.016	0.076	0.001	0.002	0.001	0.003	0.002

** Significant at P = 0.01

Data also supported the findings of Wallace and Rundel (1979), Lovenstein (1985) and Kohorn (1994) who assured the presence of some differences in leaf size and measurments between male and female jojoba plants and that females on average have larger leaves. There is also a good indication to the possibility of using leaf parameters for the pre-flowering sex determination in jojoba appeared in the researches of Chen *et al.* (1985) altnough the population size they used was limited. They used a single parameter L/W0 ratio (named L / W ratio in the published paper). Although L/W0 ratio was found not significant in the current investigation, but they found it a good tool for distinguishing males and females and they did not compare it statistically with any other parameter.

Table 2: The standard mean values of the different leaf parameters of male and female jojoba shrubs and their overall means.

Leaf parameter	Male shrubs	Female shrubs	L.S.D.$_{0.05}$	Overall mean ± S.E.
L(cm)	3.040 b	3.288 a	0.134	3.16 ± 0.12
W2 (cm)	0.939 b	1.059 a	0.059	1.00 ± 0.05
W0 (cm)	1.166 b	1.301 a	0.072	1.23 ± 0.06
W1 (cm)	0.904 b	1.067 a	0.046	0.99 ± 0.04
L/W0	2.680 N.S.	2.643 N.S.	N.S.	
W1/L	0.299 b	0.324 a	0.010	0.31 ± 0.01
W0/L	0.383 N.S.	0.394 N.S.	N.S.	
W2/L	0.308 N.S.	0.319 N.S.	N.S.	
2W1/L	0.595 b	0.644 a	0.020	0.62 ± 0.02
2W2/3L	0.213 N.S.	0.215 N.S.	N.S.	

- **Values followed by the same letters are not significantly different at 0.05 level of probability.**

2. Preflowering sex identification of unknown jojoba seedlings :

Three methods of identification were suggested and tested :

a) Preflowering sex identification by the confidence limits comparisons

In order to identify the sex of unknown eight months old jojoba seedlings, the confidence limits of the different leaf parameters of the standard known sex jojoba shrubs were first calculated (Table 3). Then the leaf parameters of 158 unknown sex seedlings were measured and scored as an average of four pairs of leaves (from the third to the sixth pair of leaves from branch top). Data of the unknown seedlings were compared with the confidence limits of the standard known sex population of Table 3. If the parameter of unknown seedling lies inside the range of the confidence limits of that parameter, the seedling sex is determined, whereas, when the parameter lies outside the confidence limits, the seedling sex is considered then as unidentifiable. According to the confidence limits comparisons (Table 4), it was found that the basic leaf parameters; L, W2, W0 and W1 were able to identify a high percentage of male seedlings (60.00, 75.00, 100.00 and 54.55% for L, W2, W0, and W1; respectively). While the indices of W1/L and 2W1/L were more effective in distinguishing female seedlings (66.67 and 75.00% for W1/A and 2W1/L; respectively). Generally, it was found that the

different leaf parameters were able to identify the seedling sex of a limited portion of the population size, not more than 21.52% in the case of 2W1/L. the efficieny of using the different leaf parameters differed from one to another, the more valuable parameters 2W1/L, W1/L, L and then W1 were successfully able to identify the seedling sex of 21.52, 20.25, 18.99 and 17.72%; respectively of the tested unknown population. While the other parameters W2 and W0 were able to identify the sex of only 11.39% of the unknown seedlings. It was noticed from the original individual data that most of the seedlings which had exceeded the upper limits of the female leaf parameters were found females although they were considered as unidentifiable because they did not statistically lie between the female confidence limits as previously stated. On the other hand, most of the seedlings which did not reach the lower limit of males were found males although being classified as unidentifiable seedlings. Therefore, a high number of unidentified seedlings were found when the confidence limits comparisons were made.

Table 3: Confidence limits of the different leaf parameters of the standard male and female jojoba shrubs.

Leaf parameters	Male shrubs	Female shrubs
L (cm)	2.95 – 3.13	3.19 – 3.38
W2 (cm)	0.90 – 0.98	1.02 – 1.10
W0 (cm)	1.16 – 1.22	1.25 – 1.35
W1 (cm)	0.87 – 0.94	1.03 – 1.10
L/W0	N.S.	N.S.
W1/L	0.29 – 0.31	0.32 – 0.33
W0/L	N.S.	N.S.
W2/L	N.S.	N.S.
2W1/L	0.58 – 0.61	0.63 – 0.66
2W2/3L	N.S.	N.S.

b) Preflowering sex identification by the overall mean comparisons

As a result of the high percentage of unidentifiable seedlings as well as the high percentage of females and males which were hidden outside the confidence limits, another identication method was developed using the overall mean of males and females for each parameter (modified after Chen *et al.*, 1985). The overall mean of any parameter was calculated as the average of male and female means of the parameter (Table 2). Since the means of males were always smaller than females, the seedling mean of any parameter was compared to the corresponding standard overall mean of that parameter. If the unknown seedling mean was greater than the standard overall mean, the seedling was considered as a female. Whereas, when the mean was smaller, the seedling was considered as a male. Individuals which have the same overall mean value of a parameter were considered unidentifiable.

According to the overall mean comparisons, it was generally found that the different leaf parameters were able to identify successfully the sex of about 53% (W1/L) to almost 66% (W1) of the unknown population of jojoba

seedlings (Table 5). Compared to the confidence limits comparisons, the percentage of unidentifiable seedlings were also greatly reduced when the overall mean method was carried out. Slight differences were found between the different leaf parameters with respect to their efficiency for the identification of seedling sex. Some parameters, however, were found highly effective in distinguishing female seedlings such as 2W1/L (79.37), W1 (73.68) and W1/L (70.00%), whereas, W2,W0,W1 and L gave good results for distinguishing male seedlings (88.89, 73.33, 68.00 and 64.29% male seedlings; respectively).

The overall mean method looked much more reliable in distinguishing male and female jojoba young seedlings not only because of the high percentage of identification compared to the confidence limits comparisons, but also because of the great advantage of assuring the seedling sex by testing more than one leaf parameter for each unknown individual.

c) Preflowering sex identification by the multiple separation method

In order to maximize the advantages (the moderate identification percentage) and to minimize the disadvantages (the high number of unidentifiable seedlings) of the confidence limits comparisons, the multiple separation method was carried out (developed after Kitat *et al.*, 1980 and Helally, 1999). The young seedling population of jojoba (158 eight months old seedlings) were first distributed according to the confidence limits of the L parameter (Table 6) which was able to separate and identify the sex of 52 seedlings (found to be 30 after flowering). The 106 remaining seedlings were considered unidentifiable according to the leaf parameter L. These 106 seedlings were redistributed according to the confidence limits of the W2 parameter. This parameter has separated and identified the sex of 22 seedlings (found to be 10 after flowering). The remaining 84 seedlings were redistributed according to the W0 parameter which identified 12 seedlings (found to be 4 after flowering). The 72 remaining were redistributed according to the W1 parameter, which identified the sex of 18 seedlings (found to be 12 after flowering). The remaining 54 seedlings were then redistributed according to the ratio W1/L which identified also 18 seedlings (found to be 14 after flowering). The 36 remaining seedlings were then finally redistributed according to the ratio 2W1/L which failed to identify the sex of any of them.

Then it was possible by using the multiple separation method to identify accurately and high reliably the sex of 70 unknown jojoba seedlings (44.30% of the tested population), 22 males and 48 females (Table 6).

Finally, if the three methods of preflowering sex identification were compared, it might be noticed that the maximum percentage of identification using the confidence limits method was 21.52% (using 2W1/L, Table 4) while it was 65.82% when the overall mean method was used (using W1, Table 5). Then, it was 44.30% in the case of the multiple separation method (Table 6). It should be then strongly recommended to use the overall mean comparisons not only due to its extremely easy way of calculations but also to the highly accurate results of sex identification as well as the high percentage of identified seedlings obtained.

Table 4: Results of preflowering sex identification of 158 eight months old jojoba seedlings using the confidence limits comparisons. Identification was carried out before flowering and confirmed after flowering.

Leaf parameter	Population size	Identified males			Identified females			Total identified seedlings				Unidentified before flowering
		Before flowering	After flowering	Accuracy %	Before flowering	After flowering	Accuracy %	Before flowering	%	After flowering	%	
L (cm)	158	20	12	60.00%	32	18	56.25%	52	32.91%	30	18.99%	106
W2(cm)	158	8	6	75.00%	24	12	50.00%	32	20.25%	18	11.39%	126
W0(cm)	158	6	6	100.00%	36	12	33.33%	42	26.58%	18	11.39%	116
W1(cm)	158	22	12	54.55%	30	16	53.33%	52	32.91%	28	17.72%	106
W1/L	158	36	12	33.33%	30	20	66.67%	66	41.77%	32	20.25%	92
2W1/L	158	24	10	41.67%	32	24	75.00%	56	35.44%	34	21.52%	102

Table 5: Results of preflowering sex identification of 158 eight months old jojoba seedlings using the overall mean comparisons. Identification was carried out before flowering and confirmed after flowering.

Leaf parameter	Population size	Identified males			Identified females			Total identified seedlings				Unidentified before flowering
		Before flowering	After flowering	Accuracy %	Before flowering	After flowering	Accuracy %	Before flowering	%	After flowering	%	
L (cm)	158	28	18	64.29%	128	78	60.94%	156	98.73%	96	60.76%	2
W2 (cm)	158	18	16	88.89%	132	86	65.15%	150	94.94%	102	64.56%	8
W0 (cm)	158	30	22	73.33%	120	80	66.67%	150	94.94%	102	64.56%	8
W1 (cm)	158	50	34	68.00%	95	70	73.68%	145	91.77%	104	65.82%	13
W1/L	158	75	42	56.00%	60	42	70.00%	135	85.44%	84	53.16%	23
2W1/L	158	82	46	56.10%	63	50	79.37%	145	91.77%	96	60.76%	13

Table 6: Results of pre-flowering sex identification of 158 eight months old jojoba seedlings using the multiple separation method, identification was carried out before flowering and confirmed after flowering.

Leaf parameter	Population size	Identified male seedlings			Identified female seedlings			Total identified seedlings				Unidentified remainder before flowering
		Before flowering	After flowering	Accuracy %	Before flowering	After flowering	Accuracy %	Before flowering	%	After flowering	%	
L (cm)	158	20	12	60.00%	32	18	56.25%	52	32.91%	30	18.99%	106
W2(cm)	106	6	4	66.67%	16	6	37.50%	22	20.75%	10	9.43%	84
W0(cm)	84	2	2	100.00%	10	2	20.00%	12	14.29%	4	4.76%	72
W1(cm)	72	2	zero	00.00%	16	12	75.00%	18	25.00%	12	16.67%	54
W1/L	54	8	4	50.00%	10	10	100.00%	18	33.33%	14	25.93%	36
2W1/L	36	zero	zero	00.00%	zero	zero	00.00%	zero	00.00%	zero	00.00%	36
Total	158	38	22	57.89%	84	48	57.14%	122	77.22%	70	44.30%	88

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دراسات على نبات الجوجوبا:
٤- التعرف على جنس الشتلات قبل التزهير.

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٢- قسم الفاكهة – كلية الزراعة – جامعة الأسكندرية.

تم إجراء هذه التجارب خلال الفترة من ١٩٩٨ إلى ٢٠٠٢ بمحطات بحوث مركز تنمية الصحراء التابع للجامعة الأمريكية بالقاهرة.

من أجل التعرف على جنس الشتلات قبل مرحلة التزهير، تم قياس وحساب بعض المقاييس والقيم الخاصة بشكل الأوراق وذلك باستخدام مجموعة شجيرات هوهوبا معلومة الجنس. تتضمن تلك القياسات طول النصل (L) والعرض عند ٤/١ طول النصل من القمة (W2) والعرض عند أعرض جزء في وسط النصل (W0) والعرض عند ٤/٣ طول النصل من القمة (W1) بالإضافة إلى ست قيم تم اقتراحها وتشمل 2W2/3L, 2W1/L, W2/L, W0/L, W1/L, L/W0. ووجد أنه يمكن استخدام ستة فقط من تلك المقاييس للتعرف على جنس الشتلات، حيث ثبت إحصائيا معنويتها وهي: W2, Wو W0 , L , W1/L وكذلك 2W1/L في حين تم استبعاد الباقين حيث ثبت إحصائيا عدم أهميتهم معنويا في التفرقة بين الإناث والذكور. أما بالنسبة لأزواج الأوراق، فقد ثبت أنه لا يوجد فروق معنوية بين الأزواج المدروسة (من الزوج الثالث حتى السادس من قمة الفرع)، مما أعطى انطباعا عن إمكانية استخدام أي زوج من الأوراق كمصدر للمقاييس الورقية السابق الإشارة إليها. أشارت النتائج إلى إمكانية استخدام المقاييس السابقة للأوراق للتعرف على الذكور والإناث في شتلات الهوهوبا. وقد تم استخدام ثلاث طرق للتعرف المبكر على جنس الشتلات :

١- التعرف على الجنس قبل مرحلة التزهير باستخدام مقارنات حدود الثقة:

من أجل التعرف على الجنس في شتلات عمرها ثماني شهور مجهولة الجنس، تم حساب حدود الثقة لجميع المقاييس الورقية من العشيرة القياسية معلومة الجنس. كذلك تم قياس نفس المقاييس الورقية لحوالي ١٥٨ شتلة هوهوبا مجهولة الجنس كمتوسط للأربع أزواج من الأوراق. وأجريت مقارنة البيانات المتحصل عليها من الشتلات المجهولة بالقيم المقارنة لها من حدود الثقة للعشيرة المعلومة. هذا وقد أوضحت الدراسة إمكانية التعرف على جنس جزء محدود من الشتلات المجهولة لا يتعدى ٢١,٥٢٪ من العشيرة المدروسة وذلك باستخدام المقياس 2W1/L.

٢- التعرف على الجنس قبل مرحلة التزهير باستخدام مقارنات المتوسط العام :

كنتيجة للنسبة المرتفعة من الشتلات التي لم تستطع طريقة حدود الثقة التعرف على جنسها وكذلك بسبب النسبة المرتفعة من الذكور أو الإناث المختفية خارج حدود الثقة المحسوبة، تم تطوير طريقة جديدة للتعرف المبكر على الجنس تتمثل في حساب المتوسط العام للإناث والذكور لكل صفة أو مقياس من المقاييس الورقية. وحيث أن متوسطات الذكور ثبت أنها باستمرار أقل من متوسطات الإناث لجميع المقاييس الورقية المحسوبة، تم مقارنة متوسط الصفة أو المقياس المحسوب لأي شتلة مجهولة الجنس بقيمة المتوسط العام القياسية للأفراد معلومة الجنس. فإذا كان متوسط الشتلة المجهولة أكبر من المتوسط العام القياسي يتم اعتبار أن الشتلة مؤنثة. أما إذا انخفض متوسط الشتلة عن المتوسط العام القياسي يتم اعتبار أن الشتلة مذكرة. أما الشتلات المجهولة الجنس ذات المتوسط المحسوب المساوي للمتوسط القياسي للعشيرة المعلومة فقد تم تسجيلها على أنها شتلات غير محددة الجنس. وطبقا لطريقة المتوسط العام، وجد عموما أن القياسات الورقية المختلفة تمكنت بنجاح من اكتشاف الجنس لحوالي ٥٣٪ من الشتلات المجهولة عند استخدام المقياس W1/L وإلى ٦٦٪ تقريبا عند استخدام المقياس W1.

٣- التعرف على الجنس قبل مرحلة التزهير باستخدام طريقة الفصل المتعدد:

باستخدام طريقة الفصل المتعدد تم التعرف على جنس ٧٠ فقط من شتلات الهوهوبا المجهولة الجنس أي بنسبة ٤٤,٣٠٪ من العشيرة المدروسة، منهم ٢٢ شتلة مذكرة و٤٨ شتلة مؤنثة. وعموما ننصح بدرجة كبيرة باتباع طريقة مقارنات المتوسط العام ليس فقط بسبب السهولة المطلقة في الحسابات ولكن أيضا بسبب الدقة العالية في التعرف على الجنس وكذلك بسبب ارتفاع نسبة التعرف على الشتلات المجهولة.