

# Breeding of *Leucospermum* to Obtain Clayey and / or Alkaline-clayey Soil Tolerance Hybrids



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## INTRODUCTION

- **The development of protea cultivation in the Canary Islands, has been based in recent years on cultivars of *Leucospermum*, such as ‘High Gold’, Tango and ‘Succession II’. The expansion of crops in areas climatologically suitable for growing these plants, presents difficulties due to the presence of clayey and /or alkaline-clayey soils.**
- **Apart from the use of rootstocks tolerant to this type of soil, tolerant hybrids would also facilitate the expansion of the crop.**



- **In 2012 a program for the production of hybrids with tolerance of those types of soils was started using as parents three pincushions tolerant to clayey and / or alkaline-clayey soils: *L. patersonii*, *L. 'High Gold'* and *L. 'Spider'* (Malan, 2012; Rodríguez-Pérez, 2017), that crossed each other and with cultivars available at that time: *Leucospermum cordifolium* 'Vlam', *L. 'Succession II'*, *L. 'Tango'* and *L. 'Veldfire'*.**



**Here you can see some photos of the species  
and cultivars used as rootstock**



***L. 'High Gold'***



***L. 'Spider'***



***L. patersonii***





- **Cultivars available at that time,  
used for crossbreeding:**



***L. 'Vlam'***



***L. 'Succession II'***



***L. 'Tango'***



***L. 'Vedfire'***



## **MATERIAL AND METHODS**

- **The assays were carried out at the Escuela T.S. de Ingeniería Agraria, (currently Higher Polytechnic School of Engineering, Agricultural Engineering Section) University of La Laguna, Tenerife, Canary Islands, Spain (28° 29'N).**
- **The procedure followed to carry out the crosses was as follows:**



- **Day 1:**
- **Select an inflorescence in which they have finished opening a few florets.**
- **Eliminate the styles of these open flowers.**
- **Cover the inflorescence with a 0.5 kg paper bag and write the date on the bag**



- **Day 2:**
- **Remove the paper bag at 48 hours. Clean pollen presenters from around 33 open florets with a soft cloth to remove pollen.**





- **Cut the styles of the remaining flowers with scissors.**



- **Replace the bag and add the date**



- **Day 3-5:**
- **Remove the paper bag at 24-72 hours.**
- **Collect fresh pollen from the desired donor plant.**
- **Pollinize the stigmas (stigmatic slots of the prepared florets.**
- **Replace the paper bag and write down the name of the pollen plant**
- **At 8 weeks after pollination, the inflorescences released the seeds, being able to collect.**



- **The seeds were obtained following the methodology proposed by Brits (1984), in which florets from the inflorescence of the plant used as mother are manually pollinated with pollen from florets from those used as father.**



- **Fourteen crosses were performed. The most recently reflexed presenters were removed from the pollen donor flowers (male parent) and used to apply the pollen directly to the stigmatic grooves of 33 reflexed florets per head of the female parent.**





- **Then the flower heads were covered with bags. Seeds were harvested about eight weeks following pollination and stored at 5°C until the following autumn.**



- **Seeds were sown at the beginning of December:**
- **The seeds were immersed in water and those that floated were discarded**





- **Floating seeds were recorded and considered unviable. Then all the seeds were rinsed with tap water and the soft gelatinous husks were removed.**



**Then they were  
scarified with full  
strength sulphuric acid  
for six minutes,  
rinsed with tap  
and distilled water.**







**Finally soaked in a  
200 ppm promalin  
solution for 24 hours.**





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**Germinated seeds in the test trays**



- **After that, seeds were dried and immersed in a Thiram (50% a.i.) solution ( $2 \text{ g L}^{-1}$ ) for half an hour. Then they were sown in a mixture of volcanic ash and peat-moss (1:1 v/v) in plastic propagating trays, 9 cm deep. These were placed in a well-ventilated greenhouse, with 50% reduction of natural light.**



- **Germinated seeds (those with both cotyledons emerged) were counted daily from the second week after sowing until the end of April 2013. Percentage germination was calculated during the trial and compared with that expected considering the viable seeds. Precocity was also determined (days till the first seed germinated).**

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- **RESULTS AND DISCUSSION**
- **As it is not possible without DNA analysis to quantify the influence of artificial selection on the resulting genome composition of the hybrids, Leonhardt et al. (2005) proposed that the percentages of species genomes in hybrids were arithmetically derived (Table 3).**



- **To identify the contributions from the different species, we used the same criterion of 50% species influence in hybrid genomes as they employed (Leonhardt et al., 2005, 2008).**



- **Almost all the crosses were successful. Seed production was in general very low. The number of seeds per flower (inflorescence) varied from 1.3 on crossing *L.* ‘High Gold’ × *L.* ‘Spider’ to 18 for *L.* ‘Spider’ × *L. patersonii* (Table 1), in agreement with that reported by Brits (1984).**



**Table 1 Crosses carried out in *Leucospermum*, showing success rate, the numbers of seeds obtained and seeds per flower.**

Cross	No. of crosses	Successful crosses %	No. of seeds	Seeds per flower
L. 'High Gold' × L. 'Tango'	3	100	5	1.7
L. 'High Gold' × L. 'Spider'	3	100	4	1.3
L. patersonii × L. 'High Gold'	2	100	9	4.5
L. patersonii × L. 'Spider'	2	100	8	4
L. patersonii × L. 'Succession II'	2	50	6	6
L. patersonii × L. 'Tango'	12	83.3	24	2.4
L. patersonii × L. 'Veldfire'	1	100	4	4
L. 'Spider' × L. cordifolium 'Vlam'	2	100	12	6
L. 'Spider' × L. patersonii	2	100	36	18
L. 'Spider' × L. 'High Gold'	1	100	8	8
L. 'Spider' × L. 'Tango'	8	100	64	8
L. 'Spider' × L. 'Veldfire'	1	100	9	9
L. 'Succession II' × L. patersonii	3	100	15	5
L. 'Succession II' × L. 'Spider'	2	50	3	3





- **That author observed on crossing several species of *Leucospermum* that seed production amounted to less than 10% of the pollinated florets, with a maximum of 38%.**



- **Table 2**
- **Shows the seed germination results and precocity.**
- **The expected germination ranged between 29.2% for *L. patersonii* × *L.* ‘Tango’ and 100% for *L.* ‘High Gold’ × *L.* ‘Spider’, *L. patersonii* × *L.* ‘Veldfire’ and *L.* ‘Succession II’ × *L.* ‘Spider’.**



**Table 2. Crosses carried out in *Leucospermum* showing the seeds obtained, viable seeds, expected and observed germination and precocity**

Cross	No. of seeds	No. of viable seeds	Expected germination %	Observed Germination %	Precocity days
L. 'High Gold' × L. 'Tango'	5	4	80	60	19
L. 'High Gold' × L. 'Spider'	4	4	100	100	16
L. patersonii × L. 'High Gold'	9	7	77.8	11.1	41
L. patersonii × L. 'Spider'	8	7	87.5	0	-
L. patersonii × L. 'Succession II'	6	3	50	33.3	16
L. patersonii × L. 'Tango'	24	7	29.2	16.7	21
L. patersonii × L. 'Veldfire'	4	4	100	0	-
L. 'Spider' × L. cordifolium 'Vlam'	12	9	75	58.3	16
L. 'Spider' × L. patersonii	36	34	94.4	33.3	27
L. 'Spider' × L. 'High Gold'	8	7	87.5	62.5	19
L. 'Spider' × L. 'Tango'	64	62	96.9	96.9	19
L. 'Spider' × L. 'Veldfire'	9	6	66.7	66.7	19
L. 'Succession II' × L. patersonii	15	5	33.3	33.3	19
L. 'Succession II' × L. 'Spider'	3	3	100	0	



- **While the observed germination ranged from 0% by *L. patersonii* × *L.* ‘Spider’ and *L. patersonii* × *L.* ‘Veldfire’ to 100% by *L.* ‘High Gold’ × *L.* ‘Spider’. Germination percentages of 42.8% of the crosses were between 58.3% and 100%.**



**Table 2. Crosses carried out in *Leucospermum* showing the seeds obtained, viable seeds, expected and observed germination and precocity**

<b>Cross</b>	<b>No. of seeds</b>	<b>No. of viable seeds</b>	<b>Expected germination %</b>	<b>Observed Germination %</b>	<b>Precocity days</b>
<b>L. 'High Gold' × L. 'Tango'</b>	5	4	80	60	19
<b>L. 'High Gold' × L. 'Spider'</b>	4	4	100	100	16
<b>L. patersonii × L. 'High Gold'</b>	9	7	77.8	11.1	41
<b>L. patersonii × L. 'Spider'</b>	8	7	87.5	0	-
<b>L. patersonii × L. 'Succession II'</b>	6	3	50	33.3	16
<b>L. patersonii × L. 'Tango'</b>	24	7	29.2	16.7	21
<b>L. patersonii × L. 'Veldfire'</b>	4	4	100	0	-
<b>L. 'Spider' × L. cordifolium 'Vlam'</b>	12	9	75	58.3	16
<b>L. 'Spider' × L. patersonii</b>	36	34	94.4	33.3	27
<b>L. 'Spider' × L. 'High Gold'</b>	8	7	87.5	62.5	19
<b>L. 'Spider' × L. 'Tango'</b>	64	62	96.9	96.9	19
<b>L. 'Spider' × L. 'Veldfire'</b>	9	6	66.7	66.7	19
<b>L. 'Succession II' × L. patersonii</b>	15	5	33.3	33.3	19
<b>L. 'Succession II' × L. 'Spider'</b>	3	3	100	0	



- **Precocity varied from 16 days for *L.* ‘High Gold’ × *L.* ‘Spider’, *L. patersonii* × *L.* ‘Succession II’ and *L.* ‘Spider’ × *L. cordifolium* ‘Vlam’ to 41 days for *L. patersonii* × *L.* ‘High Gold’. Most of the crosses showed a precocity of 19 days.**

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**Table 2. Crosses carried out in *Leucospermum* showing the seeds obtained, viable seeds, expected and observed germination and precocity**

<b>Cross</b>	<b>No. of seeds</b>	<b>No. of viable seeds</b>	<b>Expected germination %</b>	<b>Observed Germination %</b>	<b>Precocity days</b>
<b>L. 'High Gold' × L. 'Tango'</b>	<b>5</b>	<b>4</b>	<b>80</b>	<b>60</b>	<b>19</b>
<b>L. 'High Gold' × L. 'Spider'</b>	<b>4</b>	<b>4</b>	<b>100</b>	<b>100</b>	<b>16</b>
<b>L. patersonii × L. 'High Gold'</b>	<b>9</b>	<b>7</b>	<b>77.8</b>	<b>11.1</b>	<b>41</b>
<b>L. patersonii × L. 'Spider'</b>	<b>8</b>	<b>7</b>	<b>87.5</b>	<b>0</b>	<b>-</b>
<b>L. patersonii × L. 'Succession II'</b>	<b>6</b>	<b>3</b>	<b>50</b>	<b>33.3</b>	<b>16</b>
<b>L. patersonii × L. 'Tango'</b>	<b>24</b>	<b>7</b>	<b>29.2</b>	<b>16.7</b>	<b>21</b>
<b>L. patersonii × L. 'Veldfire'</b>	<b>4</b>	<b>4</b>	<b>100</b>	<b>0</b>	<b>-</b>
<b>L. 'Spider' × L. cordifolium 'Vlam'</b>	<b>12</b>	<b>9</b>	<b>75</b>	<b>58.3</b>	<b>16</b>
<b>L. 'Spider' × L. patersonii</b>	<b>36</b>	<b>34</b>	<b>94.4</b>	<b>33.3</b>	<b>27</b>
<b>L. 'Spider' × L. 'High Gold'</b>	<b>8</b>	<b>7</b>	<b>87.5</b>	<b>62.5</b>	<b>19</b>
<b>L. 'Spider' × L. 'Tango'</b>	<b>64</b>	<b>62</b>	<b>96.9</b>	<b>96.9</b>	<b>19</b>
<b>L. 'Spider' × L. 'Veldfire'</b>	<b>9</b>	<b>6</b>	<b>66.7</b>	<b>66.7</b>	<b>19</b>
<b>L. 'Succession II' × L. patersonii</b>	<b>15</b>	<b>5</b>	<b>33.3</b>	<b>33.3</b>	<b>19</b>
<b>L. 'Succession II' × L. 'Spider'</b>	<b>3</b>	<b>3</b>	<b>100</b>	<b>0</b>	



- **In general, the crosses in which *L. formosum* and *L. tottum* (parents of *L. 'Spider'*) participated ( *L. 'Spider'* × *L. 'High Gold'*, *L. 'Spider'* × *L. patersonii*, *L. 'Spider'* × *L. 'Tango'* and *L. 'Spider'* × *L. 'Veldfire'*) gave the highest number of seeds per flower (8-18), followed by crosses involving *L. patersonii*, with 50% or more (Table 3).**



**Table 3. Genome composition, by relative species percentage, for the crosses carried out in *Leucospermum*.**

Leucospermum species <sup>1</sup> :	gla.	con.	cord	tot.	lin.	pat.	for.
<b>Crosses</b>				%			
L. 'High Gold' × L. 'Tango'	25		25		25	25	
L. 'High Gold' × L. 'Spider'			25	25		25	25
L. patersonii × L. 'High Gold'			25			75	
L. patersonii × L. 'Spider'				25		50	25
L. patersonii × L. 'Succession II'			25		25	50	
L. patersonii × L. 'Tango'	25				25	50	
L. patersonii × L. 'Veldfire'	25	25				50	
L. 'Spider' × L. cordifolium 'Vlam'	25	25		25			25
L. 'Spider' × L. patersonii				25		50	25
L. 'Spider' × L. 'High Gold'			25	25		25	25
L. 'Spider' × L. 'Tango'	25			25	25		25
L. 'Spider' × L. 'Veldfire'	25	25		25			25
L. 'Succession II' × L. patersonii			25		25	50	
L. 'Succession II' × L. 'Spider'			25	25	25		25

<sup>1</sup>gla. = *L. glabrum*; con. = *L. conocarpodendron*; cord. = *L. cordifolium*;  
tot. = *L. tottum*; lin. = *L. lineare*;  
pat. = *L. patersonii*; for. = *L. formosum*



- **Rourke (1972) divided the genus *Leucospermum* into taxonomic sections that include species with floral structures more similar to each other than to species in other sections, suggesting a closer genetic relationship (Leonhardt et al., 2005).**



- **The seven species used in the crosses are classified into three taxonomic sections within the genus (Table 4). *Leucospermum cordifolium*, *L. lineare*, *L. patersonii* and *L. tottum* are included in the section Brevifilamentum, *L. conocarpodendron* and *L. glabrum* in the section Conocarpodendron, and *L. formosum* in the section Cardinitylus.**





**Table 4. Genome composition, by relative sectional percentage, for the crosses carried out in *Leucospermum*.**

Sections:	<b>Brevifilamentum</b>	<b>Conocarpodendron</b>	<b>Cardinistylus</b>
<b>Crosses</b>		%	
<b>L. 'High Gold' × L. 'Tango'</b>	<b>75</b>	<b>25</b>	
<b>L. 'High Gold' × L. 'Spider'</b>	<b>75</b>		<b>25</b>
<b>L. patersonii × L. 'High Gold'</b>	<b>100</b>		
<b>L. patersonii × L. 'Spider'</b>	<b>75</b>		<b>25</b>
<b>L. patersonii × L. 'Succession II'</b>	<b>100</b>		
<b>L. patersonii × L. 'Tango'</b>	<b>75</b>	<b>25</b>	
<b>L. patersonii × L. 'Veldfire'</b>	<b>50</b>	<b>50</b>	
<b>L. 'Spider' × L. cordifolium 'Vlam'</b>	<b>75</b>		<b>25</b>
<b>L. 'Spider' × L. patersonii</b>	<b>75</b>		<b>25</b>
<b>L. 'Spider' × L. 'High Gold'</b>	<b>75</b>		<b>25</b>
<b>L. 'Spider' × L. 'Tango'</b>	<b>50</b>	<b>25</b>	<b>25</b>
<b>L. 'Spider' × L. 'Veldfire'</b>	<b>25</b>	<b>50</b>	<b>25</b>
<b>L. 'Succession II' × L. patersonii</b>	<b>100</b>		
<b>L. 'Succession II' × L. 'Spider'</b>	<b>75</b>		<b>25</b>



- **In general, most crosses have a higher percentage of species belonging to the Brevifilamentum section, also producing the highest number of seeds per flower (Table 4).**



- **CONCLUSIONS**

- **In developing hybrids with clayey and/or alkaline-clayey soil tolerance, the use of *L. 'Spider'* appears to be more appropriate as a parent than *L. patersonii* or *L. 'High Gold'*, owing to its greater seed production per flower and the higher germination percentages of the seeds obtained.**



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**Thanks for your attention**





**The hybrids obtained were planted in a plot of the College, but although some gave very showy flowers, their growth was not adequate, presenting short or recurved flower stems, or stems with two flower heads,**

