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Influence of Temperature, Low Nutrient Supply, and Soil Composition on Germination and the Growth of Sea Kale (*Crambe maritima* L.)

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Abstract. **Sea kale (*Crambe maritima* L.) is a wild edible plant with forgotten and undiscovered potential as a field vegetable. Its natural habitat is gravel beaches in northern Europe and the Black Sea. Three experiments were conducted to find the effect of temperature on seed germination and to determine plant growth response to organic fertilizer and soil types. Germination rates were estimated at three temperatures. Plant growth responses were conducted with application of two fertilizer concentrations [15 and 30 kg plant-available nitrogen (PAN)/ha] and by using four distinct soil types. Seeds sown at 20 and 15 °C reached a significantly greater germination rate after 32 days (48.0% and 40.4%, respectively) than seeds sown at 10 °C (16.6%). The number of days when 50% of the seeds that germinated during the experiment had germinated (T_{50}) were 12.0, 11.8, and 16.8 days for 20, 15, and 10 °C, respectively. Application of 15 or 30 kg·ha^{-1} PAN did not result in any significant differences in plant size or biomass within 2 months of growth in sandy loam, but substantial plant heterogeneity was observed. Soil composition had a significant effect ($P \leq 0.05$) on plant biomass. Plants grown in fine or loamy sand had the greatest growth and biomass. Sea kale seems to have a potential to become a field vegetable, because it grows well on other soil types than gravel. However, domestication processes of the species are required to obtain homogenous plants for future propagation.**

Sea kale (*Crambe maritima* L.) is a perennial edible halophyte belonging to the Brassicaceae family with a natural growth habitat along gravel and shingle (beach gravel consisting of large, smooth pebbles unmixed with finer material) sandy beaches in Scandinavia, the United Kingdom, and along the English Channel and the northern part of the Black Sea. It is mainly distributed on the high tide line above the water splash zone on the beach. The hydrophobic surface of the thick leaves provides high salt tolerance and the leaf thickness has been shown to increase when exposed to salt spray (400 mM) imitating exposure to sea gust (de Vos et al., 2010). This edible plant has been grown in home gardens or collected along beaches around the Victorian era (1837–1901) in England and used as a vegetable; young shoots were used as asparagus, young inflorescences as broccoli, and fresh green leaves were blanched to reduce bitterness and used like spinach (Maher, 1812). The plant is almost forgotten as a crop today, but trends and studies on local and reinvented foods have increased in the last 20 years. Collectively, this can offer sea kale a renaissance as a vegetable.

Sea kale contains important secondary metabolites, especially glucosinolates, acting as defense mechanisms against insect predation, similar to other species of the Brassicaceae family. The glucosinolates are stored in vacuoles, and when leaves are disrupted, the vacuole and adjacent myrosinase-containing cells burst and the glucosinolates are released and hydrolyzed by myrosinases to isothiocyanates. This group of chemicals is associated with cancer-preventive activity and gives Brassicaceae species the characteristic mustard-like flavor (Falk et al., 2007; Lee et al., 2008; Song and Thornalley, 2007; Verkerk et al., 2009).

Sea kale is self- or cross-pollinated (Bond et al., 2005) and in fall, when the leaves wilt, the hard flower stalks with mature siliques and seeds remain over winter (Briard et al., 2002; de Vos et al., 2010). The wind disperses the hard cork-like fruits along the coasts or into the sea water. The seeds inside the fruits are able to survive in the sea water as a result of the hard pericarp (Bond et al., 2005), which also imposes dormancy and affects germination and aging (Fusheng et al., 1998; Walmsley and Davy, 1997b). Pericarp removal and soaking in gibberellic acid, water, or NaOCl solutions are some of the methods used to overcome dormancy (Fusheng et al., 1998). Although such treatments have shown to increase germination, the side effect, especially for gibberellic acid, has been high seedling mortality (22% to 77%) caused by the surface-borne fungus *Phoma lingam*. This fungus causes black leg disease in Brassicacae species that is transmitted by fruits and remains viable up to 14 months (Lloyd, 1959).

The intake of cabbage species per capita is relatively low in the Scandinavian population (Meyer and Astrup, 2011), and sea kale could be a dietary supplement to traditional cabbage species. Most research on sea kale has been ecological studies (Bond et al., 2005; Briard et al., 2002; Walmsley and Davy, 1997a, 1997b, 1997c). A few studies describe sea kale being grown as Belgian endive (*Cichorium intybus* L.) by forcing it in dark rooms to produce etiolated shoots (Fusheng et al., 1998; Fusheng and Péron, 1998; Péron, 1989, 1990). This method is said to be the most economic and profitable, but still no commercial production occurs (Briard et al., 2002). No other uses of sea kale as a vegetable have apparently been reported. Walmsley and Davy (1997c) published an ecological study on the plant in response to substrate composition around its known habitat and fertilizer. They found the highest dry mass at shingle-dominated soils and no response to fertilizer. The plant growth response has not been studied and the growth of sea kale in less sandy soil types is apparently unknown.

The objective of this study was to examine the potential to introduce the species as a crop in agriculture by an organic approach and on other soil types than it normally thrives. We focused on seed germination ability, growth rate, and biomass production at low fertilizer levels. Most vegetable crops respond to soil types and the amount of available nutrients, but no published studies on sea kale growth responses on agricultural land are apparently available. This study includes a germination experiment with seeds without pericarp at 10, 15, and 20 °C, an organic fertilizer experiment simulating the effect on PAN application of 15 and 30 kg·ha^{-1}, and a soil type experiment based on four soil types (sandy loam, gravel, fine and loamy sand). All experiments were conducted to examine response of the plants to specific growth conditions.

Materials and Methods

Seed material. Seeds (product code 188075 delivered from Suttons Seeds, Paignton, U.K.) were stored at 5 °C until experiments began. Suttons Seeds stated they only have one producer of sea kale, located in England, and all seeds were produced in 2012.

Seed germination experiment. The seed pericarps were removed manually and seeds were sown dry in planting trays of 24 cm in diameter and 5.5 cm in height containing peat soil [Pindstrup Mosebrug No. 1 (0.650 kg·m^{-3} NPK; and 50 g·m^{-3} micronutrients), Ryomgaard, Denmark] in 2-mm depth and 15-mm density. Seeds were placed on the same shelf 2 × 2 in each cabinet for three temperatures (10, 15, or 20 °C, respectively) in three growth chambers (Conviron, PGV36, Winnipeg,

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Manitoba, Canada). There were four replicates per treatment with 50 seeds in each. The seed were exposed to 10 h light with 50 µmol·m^{-2}·s^{-1} and 85% relative humidity during the experiment. Seeds were denoted as germinated when the radicle was 2 mm or greater. A seed containing a mycelium-like structure or discoloration was denoted as infected. Germinated and infected seeds were recorded and removed to prevent further fungal or bacterial infections. The germination was recorded continuously every second to third day, starting at Day 5. The experiment started 2 May 2013 and ended 3 June 2013.

Establishment of plants. The seeds without pericarp were sown in peat soil [Pindstrup Mosebrug No. 1 (0.650 kg·m^{-3} NPK; and 50 g·m^{-3} micronutrients)] in a depth of 1 to 3 mm in seedling boxes. The boxes were placed in a greenhouse at day temperature of at least 15 °C (0700 to 2200 HR) and night temperature of 13 °C (2200 to 0700 HR) with sunlight as the light source and kept moist with tap water. They were protected with a permeable fiber cloth (Agryl P17; Novagryl, Biesheim, France) the first 10 d to protect against direct sunlight and heat. Fungus gnat larvae were prevented by watering with 0.03% VectoBac (Valent BioSciences, Libertyville, IL) (v/v) three times during the growth period. Seeds were sown 2 May 2013 to produce plants for the fertilization experiment. Seeds for the soil type experiment Repetitions 1 and 2 were sown 24 June 2013.

Fertilization experiment. The fertilization experiment was conducted in a period of 59 d from 7 June 2013 to 8 Aug. 2013 in a greenhouse with at least 15/13 °C in day/night temperatures. To mimic organic production, depleted mushroom compost based on horse manure, denoted champost, was used as fertilizer delivered 4 May 2013 by Tvedemose Champignon, Tappernøje, Denmark. Two champost samples were weighed (12.06 g and 11.6 g) supplemented with 100 mL 1 M KCl, shaken for 45 min, and analyzed (Autoanalyzer III; Bran Luebbe, Chicago, IL). The nitrate content in the solution was 104.6 ± 0.9 mg·L^{-1} and ammonia content was 1.5 ± 0.2 mg·L^{-1} and corresponded to an amount equal to PAN of 15 kg N/ha referred to as 15N and 30 kg PAN/ha referred to as 30N. A control without added champost (0 kg applied PAN/ha) was denoted 0N. Fifty-four plants per treatment (0N, 15N, and 30N) were transplanted into 12.5-L boxes (dimensions: length:width:height = 39 cm:19 cm:17 cm) meaning nine containers for each treatment with six plants in each container with an individual plant distance of 14 cm at developmental Stage 13, i.e., three true leaves, leaf pairs or whorls unfolded, according to the Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie (BBCH) plant phenological development scale for leafy vegetables not forming heads (Meier, 2001). The soil type was characterized as a Sandy loam (Rowell, 1994; USDA, 1987; Table 1).

Plant height and diameter were measured two times per week for 2 months. Additionally, leaf drop was noted and the number of plants with side shoots was counted at the end of the experiment.

Soil type experiment. The soil type experiment was conducted twice displaced by time (Repetition 1, 18 July to 15 Aug. 2013; Repetition 2, 19 Aug. to 16 Sept. 2013) by transplanting 24 plants at BBCH developmental Stage 13 into plastic pots (volume was ≈1.9 L) for each soil type containing gravel sand, fine sand, loamy sand, or sandy loam (Rowell, 1994; USDA, 1987; Table 1). The soils were collected from fields that earlier had been soil type-classified. Each soil type was manually analyzed for individual soil particle composition by measuring two samples of 100 g of dried soil for each soil type. The samples were then separated through sieves with four different mesh sizes.

All soil types were analyzed for inorganic N before addition of champost corresponding to application of 30 kg PAN/ha by mixing champost in each pot separately. For each soil type, a sample of 25 g was weighed and diluted 1:25 in distilled water and then 100 mL 1 M KCl were applied. The solutions were centrifuged for 45 min and analyzed (Autoanalyzer III; Bran Luebbe). The apparatus were rinsed with Milli-Q water after each analysis. The pots were placed in a field at Højbakkegård, Taastrup (Denmark) (lat. 55°40′10″ N; long. 12°18′32″ E) with the upper edge placed 3 to 5 cm above field surface to mimic field conditions, preventing heating and avoiding excess nutrients and water overflow in the pots. Emerging weeds were removed by hand. The pots were daily drip-irrigated with 35 mL water/pot/d.

Plant radius and leaf area were measured two times per week during the experiment. A ruler and a 25.0-cm^2 marked plot were held horizontally at the plant basis of each pot and a photograph was taken from above of each plant. Plant height and diameter were defined and measured from the soil basis to the upper leaf tip and from leaf tip to leaf tip, respectively. The ruler and plot were used as a calibration tool to determine plant radius and leaf area using the biological imaging software tool ImageJ (Schindelin et al., 2012).

Fresh weight and dry weight measurements. Plants from the fertilizer and the soil type experiment were harvested after 63 and 28 d, respectively, and fresh weight and dry weight were determined. All plants were separated in root and shoot from the plant basis and each part was measured individually. The plant parts were oven-dried for 72 h at 70 °C and dry weights were measured.

Data analysis. Data were statistically analyzed and graphically presented using Statistical R (Version 2.15, GNU Project) and Microsoft Excel (Version 11, Redmond, WA; 2003). A two-tailed F-test was used to ensure equal variances on data before statistical analysis on mean differences (± SD) was performed in *t* tests (if n < 30) and z-tests (if n > 30). A *P* value of ≤ 0.05 was set to be statistically significant.

The fit of a regression analysis was based on residual plots, data distributions, data transformations, and R^2 values in a combination (Cook and Weisberg, 1999).

Seed germination was modeled using a cumulative distribution function of the standard log-logistic distribution (Andreasen et al., 2014; Ritz et al., 2013):

$$F(t) = \frac{d}{1 + \exp[b\{\log(t) - \log(t_{50})\}]} \quad (1)$$

The upper limit parameter, *d*, denotes the proportion of seeds that germinated during the experiment out of the total number of seeds present at the beginning of the experiment and as such is a unitless probability. The parameter *b* is proportional to the slope of *F* at time *t* equal to the parameter t_{50} where 50% of the seeds that germinated during the experiment have germinated. The parameter t_{50} has the same unit as the time scale considered in the experiment. By using the model formulation, we obtain a parametric model fit for an S-shaped curve (Ritz et al., 2013).

Results

Seed germination experiment. At 32 d, the end of the germination period, the total germination was 16.6% ± 2.7%, 40.4% ± 3.5%, and 48.0% ± 3.6% for 10, 15, and 20 °C, respectively, with a significantly lower germination at 10 °C ($P \leq 0.05$) from Day 8 (Fig. 1). At both 15 and 20 °C, the daily

Table 1. The average weight in percentage ± SD for each soil fraction based on two samples for each soil type.

Soil type	Gravel >2 mm	Coarse sand >1 mm	Fine sand 0.25–1.0 mm	Very fine sand 0.071–0.25 mm	Silt/clay <0.071 mm
Fine sand	6.7 ± 0.6	17.3 ± 0.3	51.0 ± 2.3	20.2 ± 2.0	4.6 ± 0.4
Loamy sand	1.5 ± 2.1	4.1 ± 2.2	26.6 ± 1.9	40.2 ± 0.1	27.5 ± 1.6
Sandy loam	5.1 ± 1.6	2.9 ± 0.4	18.8 ± 0.3	33.2 ± 0.3	39.4 ± 2.6
Gravel sand	16.8 ± 2.4	10.2 ± 0.3	31.9 ± 2.1	35.9 ± 1.0	4.8 ± 0.2

Table 2. Estimated effects on germination of sea kale seeds at 10, 15, and 20 °C during 32 d based on Eq. 1 ± SD.[z]

Germination temp (°C)	t_{50}, time to 50% germination (days)	*d* parameter × 100 (germination %)	*b* parameter slope
10	16.8 ± 1.1	16.6 ± 2.7	–5.2 ± 1.0
15	11.8 ± 0.5	40.4 ± 3.5	–4.6 ± 0.5
20	12.0 ± 0.6	48.0 ± 3.6	–3.8 ± 0.4

[z]t_{50} is number of days in which 50% of the seeds that germinated during the experiment have germinated; d is germination % after 32 d and b is the slope.

germination rate increased significantly ($P \leq 0.05$) from Day 5 to Day 8 (Fig. 1). The germination rate at 10 °C had a 5-d delay in the onset of germination. The corresponding number of days when 50% of the seeds that germinated during the experiment have germinated (T_{50} values) were 16.8 ± 1.1, 11.8 ± 0.5, and 12.0 ± 0.6 d for 10, 15, and 20 °C, respectively (Fig. 1; Table 2).

As a result of the lower germination observed at 10 °C, it can be speculated that the non-germinated and non-infected seeds were still viable, but potentially in dormancy. The infection percentage after 32 d at 10, 15, and 20 °C were 10.0% ± 3.8%, 12.5% ± 4.1%, and 30.0% ± 4.9%, respectively. At 20 °C the infection percentage was significantly higher ($P \leq 0.05$) than at 10 and 15 °C.

Fertilization experiment. Sea kale plants used for this experiment had an average height of 62.6 ± 2.9 mm and an average diameter of 88.6 ± 5.6 mm at Day 0 (Fig. 2).

The average plant height at Day 59 was 173.2 ± 4.8 mm in treatment 15N, which was significantly lower ($P \leq 0.05$) in comparison with both 30N (200.5 ± 7.2 mm) and 0N (192.2 ± 6.2 mm) (Fig. 2A). A significant difference ($P = 0.0470$) in plant diameter was observed at Day 59 in treatments 0N and 30N with diameters of 291.0 ± 14.5 mm and 328.5 ± 11.8 mm, respectively (Fig. 2B). The average diameter of treatment 15N was 270.8 ± 10.9 mm at Day 59. No significant differences were observed for the overall growth in terms of both plant height and diameter in response to added fertilizers.

The average number of leaves dropped after 59 d in treatments 0N, 15N, and 30N were 2.6 ± 0.1, 2.6 ± 0.1, and 2.7 ± 0.1 leaves, respectively, and no significant difference was observed (data not shown). The number of plants with branching shoots was 13, 11, and 11 for 0N, 15N, and 30N, respectively (data not shown).

The results after 59 d of plant growth showed differences in shoot and root fresh weight among the treatments. Plants grown with 15N added had a significantly lower shoot fresh weight ($P \leq 0.05$) compared with plants with 30N added (Table 3). However, the values for plants with 0N added were found to be intermediate. The dry weight of the shoots was significantly lower ($P \leq 0.05$) in plants from the 15N treatment in comparison with both the 30N treatment and the control 0N (Table 3).

Soil type experiment. Interactions between the soil types and leaf area were observed (Fig. 3B). The daily average growth in leaf area after 28 d were 9.4 ± 0.7 $cm^2 \cdot d^{-1}$ in sandy loam and 6.6 ± 1.1 $cm^2 \cdot d^{-1}$ for gravel sand in Repetition 1 (data not shown) and resulted in a significant difference ($P = 0.044$; $P \leq 0.05$). The average leaf area increased 5.6 to 6.7 times in 28 d in Repetition 1 to an area between 198.8 and 265.8 cm^2 (Fig. 3A). The fine sand soil and loamy sand soil leaf area were found to be in between (Fig. 3A). In Repetition 2, the daily average growth in leaf area after 28 d was largest in plants grown in gravel sand with 5.0 ± 0.5 $cm^2 \cdot d^{-1}$ and the lowest growth response was 3.0 ± 0.5 $cm^2 \cdot d^{-1}$ in sandy loam (data not shown). The observations were significantly different ($P \leq 0.05$; data not shown). The average leaf area in Repetition 2 increased with 83.1 and 141.1 cm^2 during 28 d of growth (Fig. 3B). Plants grown in fine sand had the largest plant size increase by 5.2 times and plants grown in sandy loam had the lowest increase corresponding to 3.3 times from transplanting.

In contrast to the leaf area, no correlations between the soil types and the plant radius were observed. The average plant radius and leaf area decreased from Day 0 to 4 (Fig. 3C

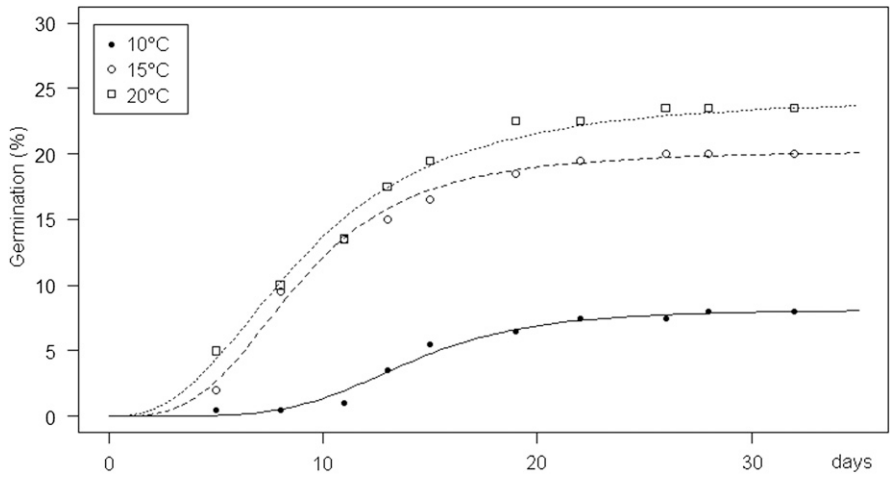

Fig. 1. Accumulated germination for seeds germinated at 10, 15, and 20 °C through 32 d. Data points are means of four replications (n = 50).

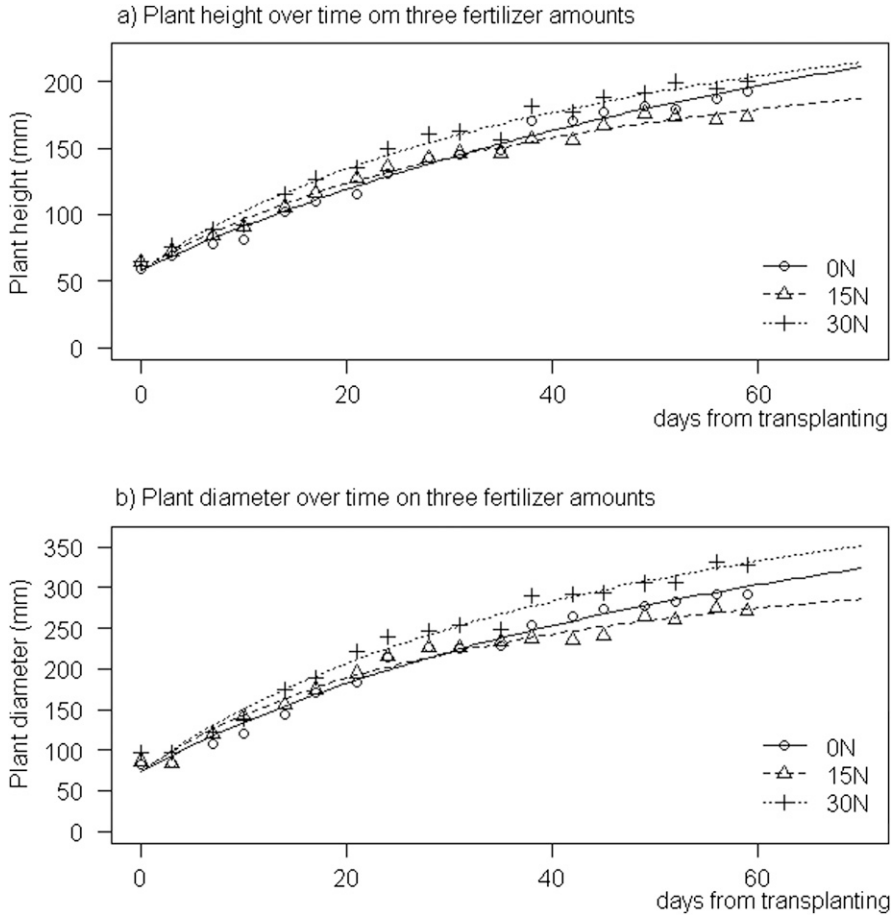

Fig. 2. Effect of champost addition on growth of sea kale. Average plant height (**A**) and average plant diameter (**B**) during 59 d. Each point represents average plant parameter (n = 54) for fertilizer treatments with no added plant-available nitrogen (PAN) (0N), addition corresponding to 15 kg PAN/ha (15N), and 30 kg PAN/ha (30N) on a sandy loam.

and D) in both repetitions. Similarly, leaf drop and wilting leaves on several plants, no matter soil types, were observed at Day 4. After 28 d, the plant radius in Repetition 1 was largest in plants grown in fine sand with 181.3 ± 7.6 mm and smallest for plants grown in gravel sand with 171.5 ± 7.8 mm (Fig. 3C), but no significant differences were found (P = 0.376). Repetition 2 resulted in the smallest radius of plants grown in sandy loam with 121.4 ± 6.9 mm and the largest radius was 139.8 ± 8.3 mm in gravel sand (Fig. 3D), but no significant differences were observed. The background PAN levels were determined for all the soil types and found in average to be ≈10 PAN/ha (data not shown).

The shoot and root fresh weights were affected by soil particle composition (Table 1). Root fresh weight in plants grown in fine sand (Repetition 1) was significantly higher compared with the other soil types in this experiment ($P \leq 0.05$) with an average weight of 17.0 ± 1.2 g (Table 4). Plants from the sandy loam had the lowest root fresh weight of 9.8 ± 1.5 g (Table 4). No significant differences were observed in root fresh weight for Repetition 2. Shoot fresh weight in plants grown in fine sand was 72.3 ± 6.0 g (Repetition 1), which was significantly higher than for gravel sand, 52.1 ± 6.8 g (Table 4); however, plants grown in gravel sand in Repetition 2 had significantly higher ($P \leq 0.05$) shoot fresh weight and shoot dry weight in comparison with fine sand and sandy loam.

Repetition 1 had an average day temperature of 24.3 ± 2.8 °C and a total precipitation of 30 mm (Fig. 3E) and Repetition 2 had an average day temperature of 20.5 ± 1.8 °C and 45 mm precipitation (Fig. 3F).

Discussion

Effect of temperature on seed germination and infection. Germination of sea kale seeds without pericarp had the highest germination at 20 °C in the chosen temperature range of 10, 15, and 20 °C (Fig. 1), but germination at 15 °C was not significantly lower and reached almost the same germination rate (≈40% germinated). Studies in *Crambe abyssinica* Hochst., which is a closely related species with similar pericarp, have shown optimum germination ≈15 to 25 °C (Deleon Martins et al., 2012; Fowler, 1991; Panno and Prior, 2009), which corresponded well to the observations in this study (Fig. 1). Moreover, number of days when 50% of the seeds that germinated during the experiment have germinated (T_{50} values) were 16.8 ± 1.1, 11.8 ± 0.5, and 12.0 ± 0.6 d for 10, 15, and 20 °C, respectively (Fig. 1; Table 2). The higher T_{50} at 10 °C indicated a delay in germination, whereas the lower T_{50} at 15 and 20 °C might be a consequence of more favorable seed germination conditions. Because no significant differences occurred in T_{50} at 20 °C and T_{50} at 15 °C, the temperature conditions to reach 50% germinated seeds were most favorable ≈15 to 20 °C. Sea kale is a wild species and has been described to have a substantial variation in germination percentage (20% to 80%), but studies have indicated up to 52% germination without pericarp removal (Fusheng et al., 1998), which is higher compared with 48.0% observed in this study after pericarp removal (Fig. 1). However, lower germination rates have also been reported (Low, 2007; Walmsley and Davy, 1997b).

The percentage of infected seeds during 32 d obtained at 10 °C was significantly lower ($P \leq 0.05$) in comparison with seeds placed

Table 3. Fresh and dry weights of sea kale shoots and roots after 59 d of growth in sandy loam with no added plant-available N (PAN)/ha (0N), champost addition corresponding to 15 kg PAN/ha (15N), and 30 kg PAN/ha (30N).[z]

	Shoot fresh wt (g)	Root fresh wt (g)	Shoot dry wt (g)	Root dry wt (g)
0N	35.7 ± 3.4 ab	4.7 ± 0.5 ab	5.0 ± 0.5 a	1.7 ± 0.2 a
15N	28.0 ± 2.5 b	4.7 ± 0.4 b	3.9 ± 0.3 b	1.7 ± 0.1 a
30N	39.6 ± 3.3 a	6.0 ± 0.5 a	5.2 ± 0.4 a	2.0 ± 0.2 a

[z]Mean values for each treatment (n = 54) ± SE. Different letters in columns are significantly different at $P \leq 0.05$.

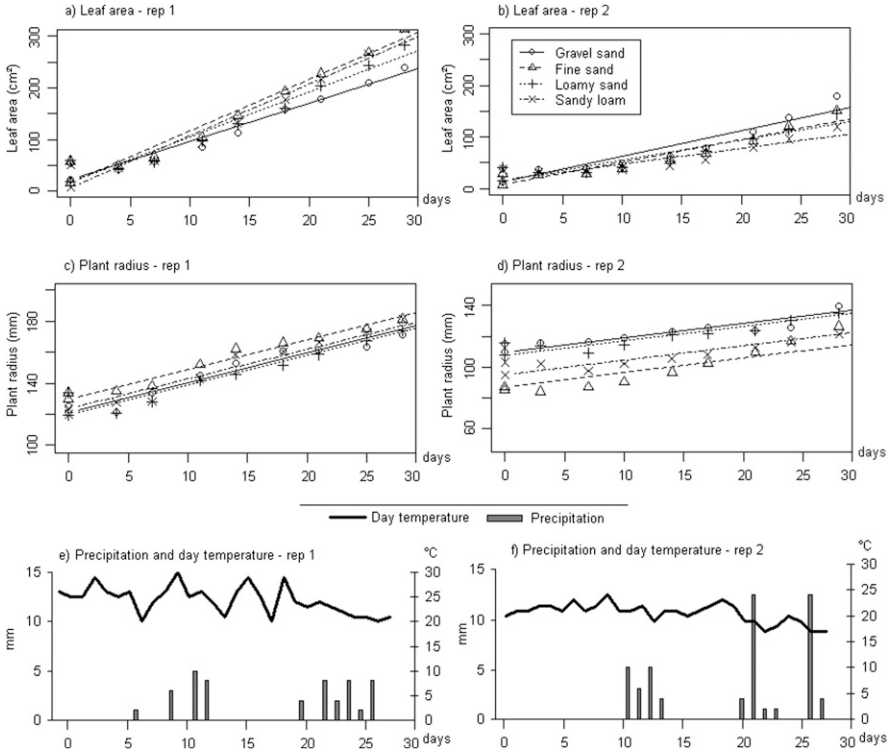

Fig. 3. Sea kale plants grown 28 d in gravel sand, fine sand, loamy sand, and sandy loam. The development of leaf area (cm²) (**A–B**) and plant radius (cm) (**C–D**) and respective regression lines. Each point represents average values of 24 plants. Precipitation and day temperature (**E–F**) in two repetitions displaced by time. Repetition 1 (rep 1) from 18 July 2013 to 15 Aug. 2013 (**A, C**) and Repetition 2 (rep 2) from 19 Aug. 2013 to 16 Sept. 2013. d = days from transplanting. Notice y-axis difference in plant radius (**C–D**).

Table 4. Average fresh and dry weights of sea kale shoots and roots ± SE growing 28 d in gravel sand, fine sand, loamy sand, or sandy loam.[z]

Repetition 1	Shoot fresh wt (g)	Root fresh wt (g)	Shoot dry wt (g)	Root dry wt (g)
Gravel sand	52.1 ± 6.8 b	11.1 ± 1.7 b	5.6 ± 0.8 a	1.7 ± 0.3 ab
Fine sand	72.3 ± 6.0 a	17.0 ± 1.2 a	7.2 ± 0.6 a	2.6 ± 0.3 a
Loamy sand	60.2 ± 6.0 ab	11.2 ± 1.3 b	6.1 ± 0.7 a	1.8 ± 0.3 ab
Sandy loam	65.0 ± 5.0 ab	9.8 ± 1.5 b	6.5 ± 0.6 a	1.6 ± 0.3 b

Repetition 2	Shoot fresh wt (g)	Root fresh wt (g)	Shoot dry wt (g)	Root dry wt (g)
Gravel sand	26.6 ± 3.0 a	5.2 ± 0.8 a	2.6 ± 0.3 a	0.6 ± 0.1 a
Fine sand	17.7 ± 2.8 b	3.8 ± 0.8 a	1.6 ± 0.3 b	0.4 ± 0.1 b
Loamy sand	19.1 ± 2.3 ab	4.6 ± 0.6 a	1.8 ± 0.3 ab	0.4 ± 0.1 ab
Sandy loam	13.4 ± 2.6 b	3.2 ± 0.6 a	1.3 ± 0.2 b	0.4 ± 0.1 ab

[z]The plants were transplanted at Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie developmental Stage 13. Repetition 1 from 18 July 2013 to 15 Aug. 2013 and Repetition 2 from 19 Aug. 2013 to 16 Sept. 2013. Different letters in columns are significantly different at $P \leq 0.05$.

at 20 °C (data not shown), which may be the result of high temperature as well as an associated increase in humidity. The infections were not analyzed, but a white mycelium-like structure emanating from the seedcoat indicated a seedborne fungi, similar to the description by Lloyd (1959) of *Phoma lingam*.

Cool stratification has earlier been reported as a method to reduce potential dormancy (de Vos et al., 2010; Low, 2007), but in this study, seeds were stored 3 weeks at 5 °C to maintain the germination quality from seed arrival until experiments began. Overcoming physiological dormancy was not part of this experiment, except pericarp removal, which is often characterized as a mechanical dormancy. Some of the non-germinated seeds used in the experiment might have been dormant.

It seems that the temperature regimes could have been the speed to obtain higher germination rates, but T_{50} indicated that the speed to maintain 50% germination was similarly reached ≈15 to 20 °C. Infections would probably increase even further at increasing temperatures. According to this study, the temperature increased both seed germination and infection, but seeds placed at 15 °C seemed to maintain a lower level of infected seeds without reduction in seed germination. If infections or humidity at 20 °C can be controlled or inhibited, then it will be favorable for germination. However, once homogenous plant material would be obtained, the plant will probably be seeded directly in the soil. Hence, it can be speculated that the temperature will affect differently than in our controlled conditions.

Effect of fertilization. Surprisingly, the growth responses for plants grown in sandy loam for 59 d with addition of champost corresponding to 15N were generally lower than control plants grown without added champost (0N) (Fig. 2). However, this difference was not significant and, moreover, plants grown in sandy loam with champost addition corresponding to 30N had the largest responses within 15 d for most of the parameters. These differences might be a result of sea kale being a species with low response to fertilizer in general. However, the heterogeneous plant size at transplanting (Day 0) might have affected the growth response, which is the result of no previous domestication or breeding of the species. The plant growth for individual plants seemed to be more or less affected by fertilization (data not shown), which should be taken into consideration in further domestication of sea kale. The results were similar to a study in which fertilizer tablets of 14N–4P–6K were placed close to the sea kale roots (Walmsley and Davy, 1997c). That study did not find evidence for fertilization demand in sea kale. Increased branching could be an advantage for a leaf potential vegetable; however, no significant effects in branching were observed (data not shown). The fresh weight and dry weight had the same pattern as the plant responses in fertilizer amounts. A domestication process might increase its requirement for fertilization; hence, selection of plants with high biomass production and low nutrient requirement is important.

Effect of soil type. The soil composition had a significant effect on sea kale biomass and leaf growth, but plant radius was unaffected in any of the two repetitions (Fig. 3). The decreased plant size at Day 4 in both repetitions was the result of wilted leaves, most likely a response to transplanting (data not shown). The highest growth response was displayed in Repetition 1 by plants grown 28 d in sandy loam (9.4 ± 0.7 $cm^2 \cdot d^{-1}$). In contrast, the lowest growth response (3.0 ± 0.5 $cm^2 \cdot d^{-1}$) and biomass (13.4 ± 2.6 g) were observed in Repetition 2 (Table 4). Moreover, plants grown in gravel sand had the lowest biomass (52.1 ± 6.8 g) in Repetition 1 but obtained the highest biomass (26.6 ± 3.0 g) in Repetition 2 compared with the other soil types (Table 4). Plant growth responses and biomass in Repetition 1 were higher, although the plants were transplanted at the same BBCH developmental stage and grown for the same duration of time. This demonstrated that displacement in time had an effect and these differences are likely explained by factors such as precipitation, temperature, and light conditions (Fig. 3E and F). The precipitation for Repetition 1 was 30 mm in total for the growth period and the average day temperature was 3.8 °C warmer than Repetition 2 (Fig. 3E and F). Repetition 1 also received higher solar irradiation (data not shown), causing elevated evaporation. Repetition 2 had a higher root water content in comparison with Repetition 1 (data not shown) and root–water relations apparently influenced shoot biomass, besides the fact that different soil compositions have different water-holding capacities and drainage capability effect. The different soils were all supplemented with 30N and background PAN levels were ≈10 PAN/ha in average (data not shown). Hence, it is not likely that the observed growth differences are effects of varying levels of background PAN among the different soil types. A study covering the natural soil amplitude of sea kale found that it performed better in shingle-predominated soils than sandy soils (Walmsley and Davy, 1997c). The current study has demonstrated growth responses opposing the plant's natural amplitude and found that it is able to thrive under these conditions. Plants grown in loamy sand and fine sand had the most stable biomass production in the two repetitions displaced by time and are the most consistent soil compositions for growing sea kale in this study.

Collectively, sea kale represents a plant of high nutritional value suited for cultivation in marginal soils. The plant tolerates low levels of nutrients and endures high levels of salt, e.g., in terms of sea fog. In domestication, it seems to be less demanding in terms of soil composition compared with its natural ecological amplitude. However, domestication processes are needed to generate uniform plant material before commercial aspects will be feasible.

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